MICROBIOLOGICAL DEPOSITION OF MANGANESE IN FRESHWATER DISTRIBUTION SYSTEMS

by

PETER ALFRED TYLER, B.Sc.(Hons.)

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DECLARATION

I hereby certify that, except as stated herein, this thesis contains no material which has been accepted for the award of any other degree or diploma in any university, and that, to the best of my knowledge and belief, this thesis contains no copy or paraphrase of material previously published or written by another person, except where due reference is made in the text.

P A Tyler

October 11th. 1967

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CONTENTS

(CONTENTS - continued)

(CONTENTS - continued)

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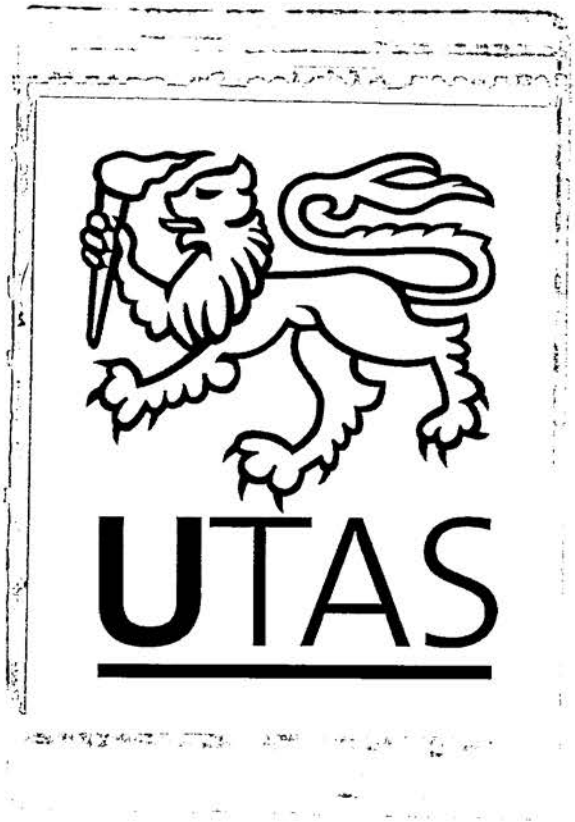
(CONTENTS - continued) Page No.



A. SUMMARY

The occurrence, in pipelines carrying freshwater, of a deposit containing 20-40% manganese, has been investigated in Tasmania. Heavy deposits form rapidly in pipelines carrying Lake King William water while those carrying Great Lake water remain free from deposits over long periods.

An apparatus was designed for studying the problem on a laboratory scale and it was proved experimentally that the manganese deposits are initiated by microorganisms. The lack of deposits in pipelines carrying Great Lake water results from lack of available manganese in the water, since addition of soluble manganese causes a deposit to form.

Lake King William was shown to be unstratified and oxygen-saturated throughout the year so that solution of manganese by anaerobic processes in the hypolimnion is not likely in this lake. It was suggested that the soluble manganese in Lake King William originates from solution in the catchment area, probably by formation of manganese chelates with the humic waters seeping from the Gymnoschoenus plains which surround this lake. Lack of this vegetation type around Great Lake, or lack of the soil type which supports it, possibly explains the lack

of soluble manganese in that water. enrichment of

Microbiological examinations showed that the chlamydobacteria are of no significance in producing manganese deposits in Tasmania. The oxidation and deposition of manganese is attributed to a stalked, budding bacterium of the genus Hyphomicrobium. This organism was shown to be widely distributed in Tasmania and also to be responsible for manganese deposits in pipelines in Queensland. The organism has been isolated in pure culture and found to be very pleomorphic. It exhibits a range of variation spanning two described genera (Hyphomicrobium and Pedomicrobium) and it is suggested that the latter genus is invalid. In its ultrastructure, the organism resembles other investigated strains of Hyphomicrobium but it may differ in possessing many flagella-like or fimbrae-like appendages. The possible significance of these as organs of attachment is considered. The attachment of cells to the pipe surface is considered in terms of electrostatic attraction and production of holdfast material. Electrophoretic studies indicate that the Hyphomicrobium cells are negatively charged so that attachment by direct electrostatic attraction is unlikely.

A pipeline is considered in terms of a continuous

culture vessel in which a selective enrichment of manganese-oxidizing bacteria occurs on the internal surface. The morphology of these organisms is discussed in relation to their efficiency in colonizing the pipe surface and in oxidizing manganese. It is likely that the curious morphology and mode of reproduction of hyphomicrobia accounts for their efficiency in producing, or coexisting with, the manganese oxides they produce.

B. HISTORICAL REVIEW

1. The history of the problem of manganese in the water supply industry

The year 1906 seems to be the time at which the presence of manganese in potable waters became widely recognised as a serious problem for water engineers. In that year, flooding of the Oder River Valley caused the manganese content of the Breslau water supply to rise to 228 mg/l, and the water became unusable. The Breslau experience was by no means the first but it was of such proportions as to focus world attention on the presence of manganese in waters (Zapffe, 1933).

Although manganese was discovered as an element in

[illegible] water utilisation has grown.

1774 (Sully, 1955), the first recorded presence of manganese in ground waters did not appear until 1896 (Zapffe, 1933). Apparently this analysis went unnoticed until the Breslau calamity in 1906 (Zapffe, 1931). However, the occurrence of deposits containing manganese in pipelines and other water-distribution systems had been observed and mentioned before 1906 (Adler, 1904; Beythien, Hempel and Kraft, 1904; Brown, 1904; Jackson, 1902; Raumer, 1903). The early reports were mostly by technical officers of water companies who were concerned principally with controlling the problem. Many of these reports are confusing and contradictory, and do not add to an understanding of the nature of the problem. None-the-less, they document the world-wide occurrence of manganese-rich deposits in pipelines and record the important fact that the manganese content of the incoming water is often beyond detection by normal analytical methods (Ingols and Wilroy, 1963; Morgan and Stumm, 1965; Myers, 1961; Wolfe, 1960).

The history of manganese problems up to 1931 has been reviewed and summarised by Zapffe (1931, 1933). Later studies have shown the problem to be widespread throughout the world and the problem of manganese deposits has become more and more an economic factor as the variety of

industrial water utilization has grown.

The undesirable effects of manganese deposits are different in different types of water utilization. In domestic water-supply engineering, the deposits interfere in several ways with the smooth operation of the industry. Early reports were concerned mainly with the clogging of sand filters, a reduction in flow by the partial blockage of fine reticulation pipes, and the dirty water caused by sloughing-off of the manganese oxides. In domestic usage this dirty water produces unpalatable water, gastro-enteritic disturbances, and permanent stains on laundered clothing and plumbing fixtures. It has caused the abandonment of traditional water supplies in many cities. On an industrial scale, the presence of manganese seriously impairs quality control in paper, textile and paint manufacture, in brewing, and in soft drink, confectionery and ice cream manufacture. In water supplies, the deposit settles on the vanes of flow meters, causing false recordings, and it also interferes with the o-tolidine test used to control chlorination. The technical literature contains very many direct references to problems of this nature (Babcock, 1951; Baylis, 1924; Beger, 1938; Griffin, 1958, 1960; Jessen, 1932; Möse and Brantner, 1966; Myers, 1961; Schilling, 1961; Waterton, 1954; Wolfe, 1960; Wolzogen-Kühr, 1927; Zapffe, 1931, 1933). The importance of the problem

can also be gauged from the voluminous technical literature dealing with removal of manganese from water supplies (e.g. Adams, 1960; Baylis, 1924; Frisk, 1932; Griffin, 1960; Vollmar, 1914; Waterton, 1954; Zapffe, 1931).

2. Manganese deposits as an economic factor in hydro-electric undertakings

In hydro-electric undertakings the occurrence of manganese deposits in the pressure pipelines poses serious economic problems of a different nature. The power produced by a power station is proportional to the net hydrostatic head between the turbine and the surface of the storage lake. This net head is the absolute hydrostatic head minus a factor "head-loss", and it is this head loss factor which is affected by manganese deposits. The presence of a deposit in a pipeline produces turbulence in the flow, so increasing the friction and the head loss (Fig. 1). Thus, there is a reduction in the power produced or, alternatively, more of the stored water must be used to maintain power output. Though manganese deposits undoubtedly have been present in many hydro-electric pipelines, the recognition of the deposits as a problem in this industry has occurred only in more recent years. There are several possible reasons

for. Next, it is often possible to clean and pipelines for inspection without interrupting power

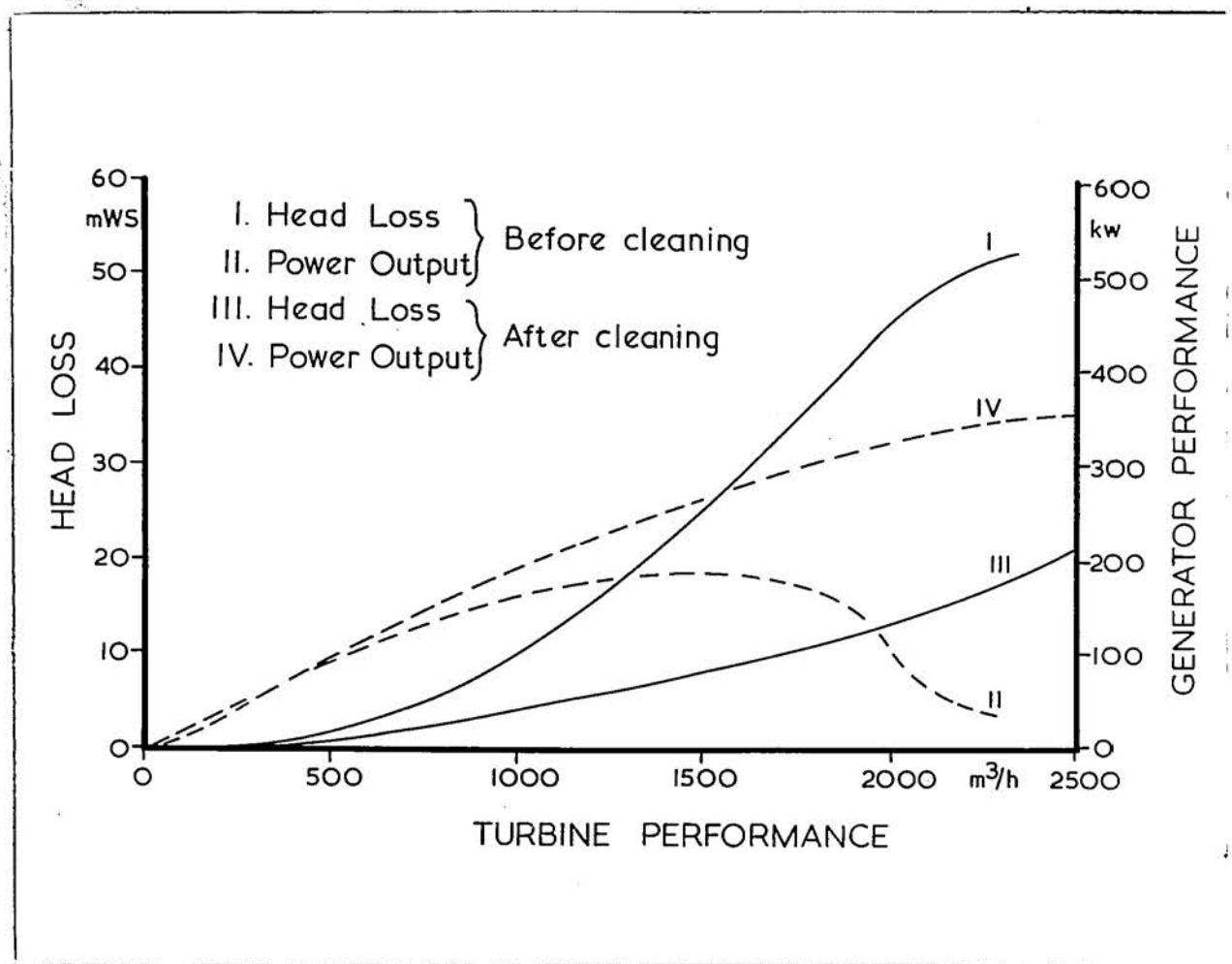


Fig. 1. Head loss of a pipeline, and power output of a generator, before and after mechanically cleaning manganese deposits from the pipeline, showing the deleterious effect of the deposits on the performance of the installation. Redrawn from Schweisfurth and Mertes (1962).

for this. First, it is seldom possible to close and empty pipelines for inspection without interrupting power supplies, so that the gradual, continuous increase in head loss, although well-known, often could not be investigated. The expense of accurate head loss determinations was also a contributary factor. Second, until recently it was not possible to fully protect pipelines against rust so that rust tubercles themselves were a source of head loss. In recent years technological advances have allowed smooth bituminous linings to give lasting protection to pipelines and this has focussed attention on biogenic manganese deposits, free of rust, as agents in increased head loss. The problem of manganese deposits in hydro-electric pipelines is referred to by Schweisfurth and Mertes (1962) and Tyler and Marshall (1967a,b).

3. The source of dissolved manganese in water supplies

In the earliest studies of pipeline deposits it was realised that the iron or manganese came from solution in the water and not from the steel of pipelines and fittings (Brown, 1904). There has been some experimental work and much speculation on the source and mechanism of solution of manganese in freshwaters. The manganese in a water supply may be derived from sediments or veins of ore in

the bottom of the reservoirs, or from the soils of the catchments through which the water percolates.

There is repeated reference to the solution of manganese from bottom deposits or veins of ore in the bottoms of the storage reservoirs, a fundamental requirement for such an event being that the lake thermally stratifies, resulting in low or zero oxygen concentrations in the hypolimnion. The solution of manganese in these situations is variously attributed to the interrelated factors of high CO_2 content, low dissolved oxygen concentration, the low redox potential and the presence of organic matter undergoing fermentative breakdown with the release of organic chelators (Adeny, 1897; Gorham, 1964; Gorham and Swaine, 1965; Hem, 1964; Hopkins and McCall, 1932; Ingols and Wilroy, 1962, 1963; Mackereth, 1966; Morgan and Stumm, 1964; Mortimer, 1941, 1942; Myers, 1961; Perkins and Novielli, 1962; Randolph, 1934; Wiedeman and Fetner, 1957). The subject is partially reviewed for freshwater by Ehrlich (1963a), Hutchinson (1957), and Silverman and Ehrhich (1964). Ingols and Wilroy (1962) found that in laboratory experiments addition of tannic acid to bacterial cultures enhanced solution of manganese and that tannins were present in waters which contained manganese. Hem (1965) has also shown the efficiency of tannic and gallic acids in bringing manganese dioxide into solution in

known and, as in Greenwood Lake, complexed form. Kjensmo (1967) describes lakes where oxygen concentrations are maintained in solution in the hypolimnion in such concentrations that the lakes are not chemically stratified (iron-manganese meromixis). In this case a supply of humic material washed in from the surrounding catchments maintains low redox potentials in the hypolimnion, partly by oxygen consumption and partly because humic acids themselves possess low redox values. The association of dissolved manganese with humic-influenced lakes is apparently a general phenomenon (Ohle, 1934; Åberg and Rodhe, 1942; Järnefelt, 1963).

4. Oxidation and reduction of manganese in soils

From the voluminous literature on forms of soil manganese it is evident that the processes of oxidation and reduction of manganese compounds also takes place in many soils, and that the factors involved broadly parallel those in freshwater. Several authors have noted that divalent manganese can be oxidized in the soil to form insoluble oxides, sometimes producing manganese deficiency in plants (Barbier and Trocme, 1950; Beijerinck, 1913a,b; Gerretsen, 1937; Leeper and Swaby, 1940; MacLachlan, 1941; Starkey, 1955; Timonin, 1950a,b). Reduction and solubilization of manganese in soils is well

known and, as in freshwater, low redox potentials, low oxygen concentrations, organic matter and chelating agents are all claimed to have a significant influence on the process (Heintze and Mann, 1946; Mandal, 1961; Starkey, 1955; Wallace, 1963; Yakubov and Vel'gorskaya, 1964). There is a cycle of manganese in soils, based on redox equilibria (Barbier and Trocme, 1950; Fujimoto and Sherman, 1948; Mann and Quastel, 1946; Reid and Miller, 1963; Sherman, McHargue and Hodgkiss, 1942; Starkey, 1955; Weir and Miller, 1962), or the interrelated factors of dissolved O_2 and fermentative breakdown of organic matter.

5. The involvement of microorganisms in deposition of manganese in pipelines

The question of whether the manganese deposit is brought about by microorganisms or whether it is a purely chemical process has been argued throughout the history of the problem in piplines. However, most authors (Baylis, 1924; Beger, 1938; van Beneden, 1955; Brown, 1904; Jackson, 1902; Jessen, 1932; Schilling, 1961; Schweisfurth and Mertes, 1962; Schweisfurth, 1963; Tyler and Marshall, 1967a,b; Vollmar, 1914; Wolfe, 1960; Wolzogen-Kühr, 1927) have attributed at least an indirect role to microorganisms and proponents of a purely physico-chemical reaction are

rare. Weston (1927) claims that the only role of bacteria is the provision of a surface onto which divalent manganese is adsorbed to be oxidized by physico-chemical means, but experimental work has shown that manganese cannot readily be precipitated by atmospheric oxidation below about pH 8.0 to 10.0 (Wolfzogen-Kühr, 1927; Waterton, 1954; Alexander, 1961; Morgan and Stumm, 1964). However, Waterton (1954) showed that a rise in water pH, upon contact with the cement lining of a pipeline, was sufficient to bring about oxidation by purely chemical means. This was, however, a special case.

Where microorganisms have been blamed as the causative agents, the sheathed bacteria (Chlamydobacteriales) almost invariably have been implicated. The history of the sheathed bacteria, which dates from 1836, is bound up with deposits of iron in pipelines and natural seepages, and it was not until 1892 that Molisch first noted manganese oxidation by this group of bacteria (Zapffe, 1933). Later it was claimed (Zapffe, 1933) that four species of sheathed bacteria could "attack" manganese, some of them doing so in preference to iron. Chlamydobacteria have been implicated as the cause of pipeline deposits by van Beneden (1955), Beger (1938), Jackson (1902), Schorler (1904), Vollmar (1914), Wolfe (1960) and Zapffe (1931, 1933). Other authors refer to the ability of these organisms to oxidise manganese without specific reference to pipelines

(Butkewitsch, 1928; Johnson and Stokes, 1966; Pringsheim, 1949a, b; Wolfe, 1963). Their taxonomy is confused, and not all the names used by earlier workers are valid (Mandel, Johnson and Stokes, 1966; Mulder, 1964; Mulder and van Veen, 1963; Pringsheim, 1949a,b). It is clear from Zapffe's review that the chlamydobacteria were held responsible in almost all cases and, indeed, early attempts to remove manganese from water supplies employed filter beds seeded with these bacteria (Zapffe, 1933).

There were workers, however, who claimed that bacteria other than chlamydobacteria were responsible for manganese deposits. Thus von Wolzogen-Kühr (1927) believed bacilli and cocci to be the principal oxidizers in his case. In more recent studies cocci and bacilli have again been implicated (Möse and Brantner, 1966; Schweisfurth and Mertes, 1962; Schweisfurth, 1963) and in the present investigation stalked, budding bacteria are considered to be the causative organisms (Tyler and Marshall, 1967a, b). Schweisfurth (1963) and Tyler and Marshall (1967a,b) find that chlamydobacteria are rare and of no importance in their deposits.

In recent years several puzzling new organisms which oxidise manganese in lake sediments have been described by Soviet microbiologists (Zavarzin, 1961b, Perfil'ev et al., 1965) including the enigmatic Metallogenium

symbioticum. However, the authenticity of at least some of these bizarre organisms is in doubt.

6. Involvement of microorganisms in the oxidation of manganese in soils

Kosegarten (1957), Mann and Quastel (1946), and Zavarzin (1962) have all shown that in soil perfusion experiments metabolic inhibitors such as sodium azide prevent oxidation of manganese by poisoning the manganese-oxidizing bacteria. Other authors (Beijerinck, 1913a,b; Bromfield, 1956; Bromfield and Skerman, 1950; Gerretsen, 1937; Leeper and Swaby, 1940; Timonin, 1950a,b) have demonstrated microbial oxidation of manganese using soil plaques or similar procedures, and various microorganisms capable of oxidizing manganese on artificial media have been isolated. However, in many cases a medium employing citrate or other hydroxyacids was used. Bromfield and Skerman (1950) showed that many microorganisms which oxidised manganese on citrate media could not do so in soil or on media free of hydroxyacids. Earlier, Söhngen (1914) had shown that hydroxyacids catalyze the autoxidation of manganous salts and that the role of microorganisms on such media was simply that of raising the pH to the optimum value for the reaction. Nevertheless, many

microorganisms have now been isolated from soils on simple media not containing hydroxyacids. Beijerinck (1913a) isolated Bacillus manganicus but Zavarzin (1962) doubts the validity of this species. Bromfield (1956) showed that strains of Corynebacterium and Chromobacterium oxidized manganese synergistically, though the Corynebacterium was the principal partner since it gave rise to a strain which could oxidize manganese in the absence of the Chromobacterium. Zavarzin (1962) isolated two strains of Pseudomonas which also oxidized manganese by an unequal synergism. Aristovskaya (1961) described oxidation of manganese by Pedomicrobium, a new stalked, budding bacterium from soils near Leningrad, and Tyler and Marshall (1967a,b) isolated a similar organism from manganese deposits in pipelines. However, pure culture studies have shown that this latter organism is a pleomorphic strain of Hyphomicrobium and that the genus Pedomicrobium is probably invalid (Tyler and Marshall, 1967c).

Manganese-oxidizing actinomycetes have been isolated from soil (Baars, 1950; Timonin, 1950b) and oxidation of manganese by soil fungi has been widely reported (Beijerinck, 1913a,b; Bromfield and Skerman, 1950; Thiel, 1925; Timonin, 1950a,b). Similar fungi have also been repeatedly isolated from manganese deposits in freshwater environments (Wolzogen-Kühr, 1927; Schweisfurth and

Mertes, 1962; Schweisfurth, 1963; Tyler and Marshall, 1967a,b). Zavarzin (1961a) claims that oxidation of manganese by fungi may be attributed to an enigmatic symbiont which he names Metallogenium symbioticum. However, the morphology and dimensions of this "organism" are so peculiar (Zavarzin, 1963) that some doubt remains as to its authenticity.

7. The role of microorganisms in oxidation of manganese

The mechanism of microbial oxidation of manganese has been the subject of much speculation and little experimentation. However, the efficiency of the mechanism is clearly demonstrated by the accumulation in pipelines of thick deposits containing up to 50% manganese even though the water flowing through the pipes may contain only minute amounts of manganese (Myers, 1961; Tyler and Marshall, 1967a; Wolfe, 1960) and, in fact, only minute amounts of any dissolved substances. Alexander (1961) and Silverman and Ehrlich (1964) divide possible mechanisms into indirect and direct categories.

(a) Indirect action

In indirect oxidation there is no enzymatic interaction,

the microorganisms bringing about oxidation by generating oxidizing conditions, or otherwise altering the environment. In this category is the catalytic autoxidation of manganous salts in media containing hydroxyacids (Söhngen, 1914). On such media, microorganisms bring about oxidation simply by raising the pH (Bromfield and Skerman, 1950). On the other hand, microorganisms may function by utilizing the organic moiety of organo-manganese chelates present in the water, thereby depositing residual manganese (Baylis, 1924; Silverman and Ehrlich, 1964). Aristovskaya (1961) found that bacteria could precipitate both iron and manganese by this method and Gruner (1922) and Harder (1919) have also suggested that chlamydobacteria may precipitate iron by removal of an organic ligand.

(b) Direct action

In direct action, microorganisms are thought to interact enzymatically with manganese compounds, either autotrophically or heterotrophically. The autotrophic oxidation of iron by Thiobacillus ferroxidans is well proven (Silverman and Lundgren, 1959) and it seems very likely that the same is true for Gallionella (Kucera and Wolfe, 1957; Sartory and Meyer, 1948. However, autotrophy has not been demonstrated in manganese-oxidizing bacteria though enzymatic oxidation of manganese by heterotrophic

microorganisms is very probable. Silverman and Ehrlich (1964) consider that the experiments with metabolic inhibitors (Mann and Quastel, 1946) provide a strong indication of such activity. Bromfield (1956) ~~found~~ suggested that an intracellular enzyme system was involved when Corynebacterium oxidized manganese. Kenten and Mann (1950) described a system in which divalent manganese was oxidized by an oxidation product of a phenolic substrate in a plant extract containing peroxidase, and Andreae (1955) postulated that manganese was oxidized by the oxidation-product of a hydrogen donor in a system containing catalase.

The question of the nutrition of Sphaerotilus discophorus has been the subject of long and controversial investigation and the question is still not solved though Skerman (1959) regards it as a facultative autotroph. Recently, Johnson and Stokes (1966) obtained oxidation of manganese by washed cell suspensions of S. discophorus and presented good evidence that oxidation is brought about by an inducible enzyme. Mulder (1964), however, claims that oxidation in this species is caused by diffusible metabolic products, and Johnson and Stokes admit that their results can be explained in terms of the inducible enzyme producing metabolic products of the type considered by Mulder. None-the-less, they consider

that the evidence weighs in favour of a direct enzymatic oxidation. [illegible] Ehrlich (1967) presents the strongest evidence for enzymatic oxidation of manganese by microorganisms. Cell free extracts of a marine manganese-nodule bacterium brought about oxidation of manganous salts. The active principle was thermolabile and susceptible to enzyme poisons.

8. The role of microorganisms in reduction and solution of manganese

As in the case of microbial oxidation of manganese both direct and indirect processes are involved.

(a) Indirect action

Many reports of microbial reduction of manganese correlate bacterial activity with organic matter and low redox potential. When organic matter accumulates in water or soils with low dissolved oxygen concentrations microbial oxidation of the organic debris lowers the oxygen and redox levels, and releases organic complexing acids. All these factors favour solution of manganese. Ingols and Wilroy (1963) consider that when a new reservoir is flooded, microbiological decomposition of the flooded vegetation leads to solution of manganese by

the factors mentioned above. Similar views are stated by Baylis (1924), Hem (1964), Hopkins and McCall (1932), Myers (1961), and Starkey (1955).

(b) Direct action

Perkins and Novielli (1962) carried out experiments in which growing bacteria successfully leached high concentrations of soluble manganese from low-grade ores, and Ehrlich (1963b) reported a similar direct bacterial leaching of marine manganese nodules. Vavra and Frederick (1952) showed that in perfusion experiments bacteria accelerated the release of divalent manganese from soils. However, in all these cases organic matter was present and essential and, although the bacteria appeared to play a direct role, it is likely that the mechanism was an incidental effect. Hochster and Quastel (1952) and Mann and Quastel (1946), however, have provided evidence that manganese can act as an alternative terminal hydrogen acceptor in place of oxygen during anaerobic bacterial respiration, thus acting directly with an enzyme system.

9. Conclusions

From the above review it is clear

a) that the oxidation and deposition of manganese in pipelines and tunnels conveying freshwaters is of

widespread occurrence, and that where such deposition occurs it gives rise to considerable problems, both in domestic and industrial water supplies.

b) that where stratification develops, manganese can enter solution from the mud or from manganiferous rocks in the beds of reservoirs, under the anaerobic conditions of the hypolimnion.

c) that similar processes of oxidation and reduction of manganese occur widely in soils, where there is a manganese cycle based on redox equilibria. Manganese solubilized in soils of lake catchments may be a source of dissolved manganese in lakes.

d) that there is strong evidence for involvement of microorganisms as agents of deposition and solution of manganese in both freshwater and soil environments.

e) that in most early reports the sheathed bacteria (chlamydobacteria) were believed to be the sole oxidizing organisms whereas some later reports have implicated other types of bacteria.

f) that the mechanism of microbial transformations of manganese is not clear but that both indirect transformations and direct, enzymatic transformations are likely, depending on circumstances.

g) that the existing literature is confused and often contradictory and, in the case of freshwater studies,

is marked by lack of continuity of study and control of experimental conditions.

In these circumstances the present investigation was with justification commenced as a broad survey of the problem in Tasmania.

C. INTRODUCTION TO THE PROBLEM IN TASMANIA

When the present investigation commenced little was known about the problem in Tasmania. It was evident that in some pipelines a deposit containing as much as 40% manganese occurred, forming a lining about 7 mm thick, and that this was responsible for significant head loss. The deposits occurred only in the Derwent pipelines, carrying waters of Lake King William (Fig. 2). After installation of new pipelines at the Tarraleah and Butler's Gorge power stations, the head loss gradually built up until it reached a maximum after about six months. When, at a later date, the Liapootah, Wayatinah and Catagunya power stations were commissioned further downstream, a similar situation developed.

In contrast to this, the Shannon and Waddamana pipelines, carrying waters of Great Lake (Fig. 2), had remained practically free of deposit over a period of 40 years.

A slight deposit was present, but it was mainly [illegible]
mud and had a low [illegible]. The plant [illegible]
Lake Echo and from the Nive river [illegible]

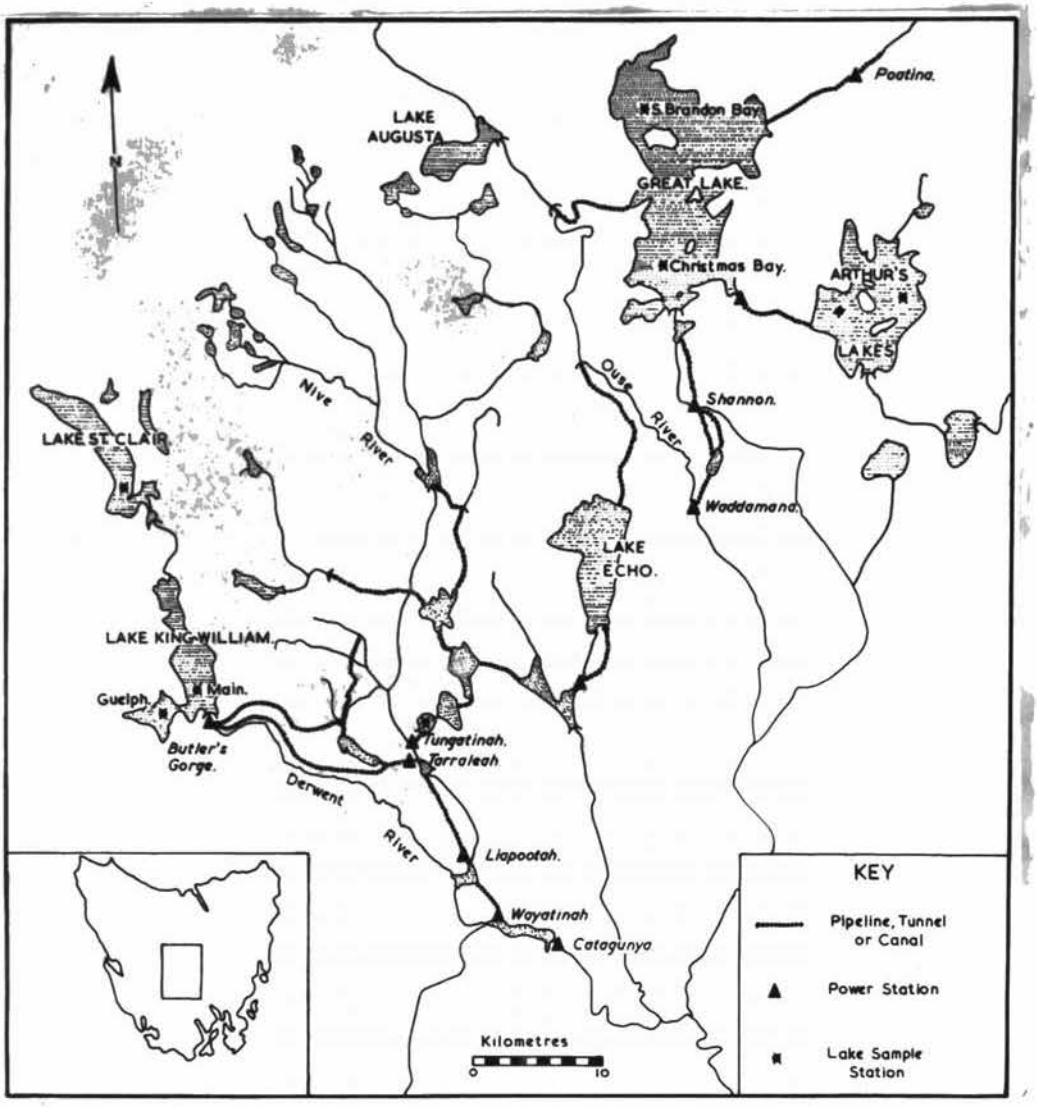


Fig. 2. Map of the Central Plateau of Tasmania, showing the location of lakes, pipelines, power stations and the biological sampling points on the lakes.

A slight deposit was present, but it was mainly organic mud and had a low manganese content. The pipelines from Lake Echo and from the Nive River had not been examined in detail but were believed to have no serious deposits. The problem in Tasmania naturally resolved itself into a comparison of the situation in the Lake King William area and the Great Lake area.

The main lakes at present utilized for generation of hydro-electricity in Tasmania are on the Central Plateau of the State, between 41.5° and 42.5° latitude South and at elevations between 714 and 1030 m (Fig. 2).

Lake King William is an artificial reservoir formed by damming the Derwent River in 1947. It lies at an elevation of 768 metres. The main arm follows the course of the flooded Derwent river and is approximately 68m deep at the downstream end. The Guelph Arm, which flooded a flat plain, is approximately 20m deep. Depths vary greatly, however, depending on inflow and outflow.

Lake St. Clair, the source of the Derwent River, is a deep glacial lake at an elevation of 793 metres. The level has been raised slightly by damming and the depth at the deepest point is now approximately 200m. Lake St. Clair and Lake King William provide the water for the Derwent system of power stations. It is in the pipelines supplying these stations that heavy deposits

of manganese occur.

Great Lake is a large, semi-natural lake at an elevation of 1030m on the Central Plateau. It has an area of approximately 150 Km^2 at full storage level and a mean depth of 10m over a flat bottom. The level was raised by damming in 1922. Until recently the water was developed southwards through the Shannon and Waddamana power stations but since 1965 Great Lake water has been utilized northwards through the new Poatina power station.

Arthur's Lakes lie to the East of Great Lake, at an elevation of 952m. They are approximately 5m deep with flat, sandy bottoms, but during the course of this investigation a dam was constructed which raised the levels and united the lakes. At the time of the investigation, Arthur's Lakes water was not being used though a plant was being installed to pump Arthur's Lakes water into Great Lake. In view of the possibility that this may produce manganese deposits in the Poatina pipelines, Arthur's Lakes were also investigated.

D. AIMS

The aims of the present investigation are

1) to survey the Tasmanian case of a manganese deposit in pipelines and to compare the storages and catchments

in the Great Lake and Lake King William areas insofar as they relate to reasons for the presence or absence of manganese deposits in the two systems.

2) to determine the nature of the manganese deposit and the agency by which oxidation and precipitation is brought about in the pipelines.

3) to prove microbial involvement, to determine the species of microorganisms concerned and their relative importance as depositors of manganese.

4) to consider the morphology and biology of the implicated microorganisms in relation to their efficiency in colonizing and dominating the pipe surface and in oxidizing manganese.

5) to consider possible mechanisms of oxidation of manganese in the pipelines and of reduction and solution of manganese in the catchments.

6) to predict the probability of troublesome deposits occurring when lakes and rivers are exploited in the future.

E. MATERIALS AND METHODS

1. Background ecology of lakes and catchments

The lakes principally investigated were Lake King William, Lake St. Clair, Great Lake, and Arthur's Lakes (Fig. 2).

Samples for chemical analysis were collected in well-rinsed polyethylene bottles. Either a surface sample was taken with a bucket or a 5m column of water was sampled with a plastic hosepipe (Lund, 1949). Precautions against decomposition or adsorption were taken according to the methods of Mackereth (1963) and American Public Health Association (APHA) (1960). Chemical analyses were carried out by the Tasmanian Government Analyst using the methods of APHA (1960).

Temperature/depth profiles of the lakes were recorded using a thermistor, and transparency was determined with a Secchi disk and water telescope (Welch, 1948). Samples for dissolved oxygen determinations were taken with a Kemmerer-type closing bottle (Welch,1948) and determined by the Alsterberg-azide modification of the Winkler method (APHA,1960).

Qualitative plankton samples were taken by towing a plankton net of 60µ pore size. Plankton was examined live with a McArthur hand microscope while other samples were fixed with 4% formalin or iodine solution for examination in the laboratory.

2. Chemical analyses of pipeline deposits

Whenever a pipeline could be opened, samples were

taken for chemical and microbiological analysis. For the latter, only the very surface of the deposit was scraped into sterile petri-dishes and returned to the laboratory under refrigeration. Sampling was carried out as soon as the pipeline had drained, before the deposits had dried noticeably.

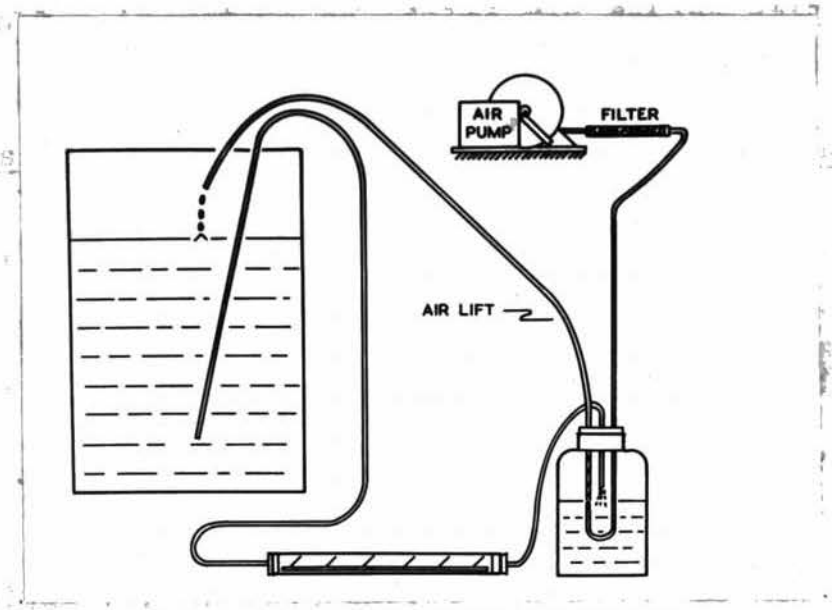
Samples for chemical analysis were dried to constant weight at 105°C and the percentage composition determined by methods based on those of APHA (1960) for water. The analyses were carried out by the Tasmanian Government Analyst.

3. Laboratory simulation of pipeline deposition

Because of the difficulty of frequent access to pipelines, simple laboratory apparatus was devised to simulate conditions in the pipelines. When certain natural waters were circulated in this apparatus, deposit containing oxidized manganese was produced. As the concentration of manganese and other ions is very low in the natural waters it was necessary to circulate a relatively large volume of water, so providing a sufficient total amount of essential elements which could be extracted from the water by bacteria adsorbed on surfaces placed in the flowstream. Water for use in these tests was

collected from the lakes in 25 litre polyethylene cans, previously rinsed with concentrated hydrochloric acid. Before sampling, the cans were well rinsed with lake water to constant pH.

The apparatus (Fig. 3) consists of a closed 250 litre polyethylene drum from the base of which water siphons and passes through 3 cm - diameter tubes into a sealed bottle. From there it is returned to the drum by an airlift. Plastic and glass tubing was used throughout to avoid having metal parts in contact with the water, and light was excluded from the system to prevent the development of algae. Removeable surfaces for inspection of deposits consisted of rows of microscope coverslips held obliquely in the flow by plastic holders. Before use, the drums were sterilized by steaming for 1 hour. All glass components were autoclaved before use and new rubber and plastic tubing used each time. When necessary, water was sterilized by autoclaving in glass containers for 1 hour at 120°C in batches of 25 litres. The presence of oxidized manganese in the deposits which formed in this apparatus was confirmed by the benzidine test (Bromfield, 1956) and by oxidizing to permanganate with sodium periodate. The equipment was used to study the mechanism of oxidation, to compare the severity of the problem in different waters and to predict the likelihood



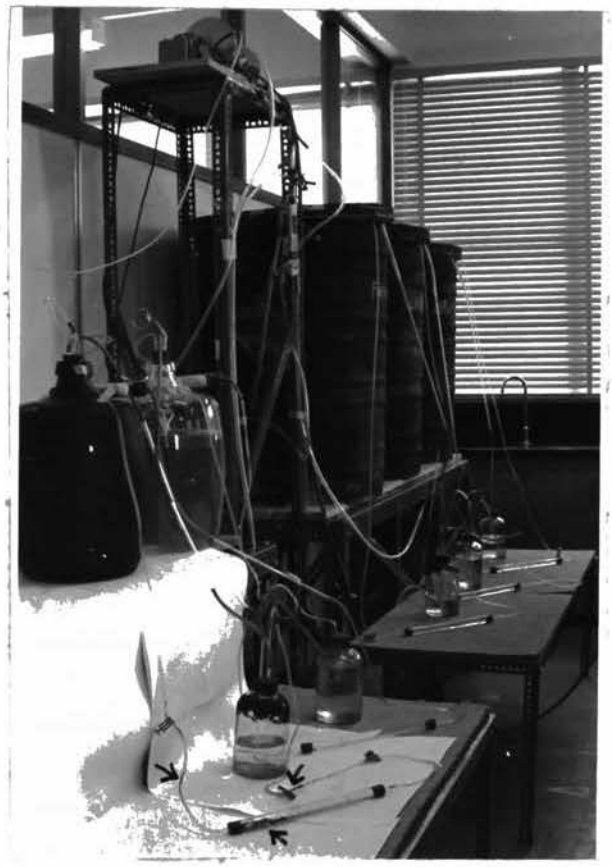


Fig. 3. The recirculatory apparatus used to study manganese deposition. Note deposits (arrowed).

of the problem in waters scheduled for future utilization.

4. Isolation of manganese-oxidizing microorganisms

Deposits from the pipelines and from the laboratory equipment were ground between two groundglass slides and plated out in dilution series on various media. Alternatively, a sterile loop was dipped into the deposits and streaked on various media. The following media were used:

a) PC Medium (after Pringsheim, 1949a) - "Difco" yeast extract, 0.05g; $MnSO_4.4H_2O$, 0.02g; "Difco" agar, 20g; tap water, 1 litre. This was used for initial platings of deposit. Manganese-oxidizing organisms were readily detected on this medium by means of the brown colonies containing manganese oxides which they produced.

b) BM Medium (after Bromfield, 1956) - KH_2PO_4, 0.05g; $MgSO_4.7H_2O$, 0.02g; $(NH_4)_2SO_4$, 0.1g; $Ca_3(PO_4)_2$, 0.1g; $MnSO_4.4H_2O$, 0.05g; "Difco" yeast extract, 0.05g; "Difco" agar, 20g; distilled water, 1 litre. This was used for the same purpose as PC medium.

c) Z1 Medium (after Zavarzin, 1961a) - $MnCO_3$, 1g; Agarose (Seravac Laboratories), 5g; tap water, 1 litre. This was used for certain fungi which oxidized manganese.

d) 337 Media. For maintenance, and sometimes for initial

isolation of hyphomicrobia, the media of Hirsch and Conti (1964) were used.

337 Medium - KH_2PO_4, 1.36g; Na_2HPO_4, 2.13g; $(NH_4)_2SO_4$, 0.5g; $MgSO_4.7H_2O$, 0.2g; $CaCl_2.2H_2O$, 0.01g; $FeSO_4.7H_2O$, 0.005g; $MnSO_4.4H_2O$, 0.2g, $NH_4MoO_4.2H_2O$, 0.0025g; "Difco" agar, 20g; distilled water, 1 litre.

337M medium - 337, with methanol vapour as carbon source.

337MH medium - 337, with incorporation of 3.37 g/l CH_3NH_2HCl as carbon source. In all 337 media phosphates were autoclaved separately and added aseptically to the cooled medium.

e) For maintenance of fungi and bacteria (other than hyphomicrobia) respectively, potato dextrose agar (PDA) and nutrient agar (NA) slants were used. Hyphomicrobia were maintained on 337MH.

As growth of various fungi frequently swamped the plates before oxidizing bacteria had time to grow, fungal development was prevented where necessary by incorporating Actidione (= Cycloheximide) in the medium at a concentration of 400 µg/ml.

5. Microscopy

Natural deposits from pipelines, and the deposits

produced in the laboratory were suspended in 5% oxalic acid to dissolve the manganese. After several washings the remaining material was examined in aqueous mounts by phase contrast microscopy or by transmitted light after staining with carbol fuchsin.

Microorganisms in pure, agar cultures were examined by squashing under the coverslip a block of agar containing the colonies. Where necessary manganese was removed with oxalic acid, and the agar by leaching with hot water.

Photomicrographs were taken by tungsten or electronic flash illumination on Ilford FP3 film developed in Agfa Rodinal at 1:15 dilution for 8 mins. at 20°C.

Some electron micrographs of thin sections were taken by Dr. Y.T. Tchan using the methods of Tchan and Webber (1966). Others were taken by the author, using the same methods plus lead-citrate staining by the methods of Reynolds (1963). Unless otherwise acknowledged, negative-stained and shadow-cast micrographs were taken by the author, using the methods of Kay (1965), under the direction of Professor A.B. Wardrop.

6. Electrophoretic studies

For electrophoretic studies of hyphomicrobia, cells from a pure culture grown on medium 337MH were washed

twice in a range of buffer solutions on ionic strength 0.015. Measurements of velocity were made over a distance of 69μ at 25°C in an assembly resembling that of Loveday and James (1957). Readings were taken on a least 10 individual cells in both directions, and the average velocity was used to calculate electrophoretic mobility. Full details of buffer compositions and calculations of mobility are given by Marshall (1967).

F. RESULTS

1. General Lake Ecology

(a) Temperature regimes

Over the lakes area, mean air temperatures range from 2°C in the winter to 11°C in summer. All the lakes are exposed and subject to frequent high winds. Under these conditions, summer lake temperatures remain relatively low and the lakes do not stratify. They appear to be thoroughly mixed throughout the year. Ice does not form except in sheltered bays. Figs. 4-11 show the temperature/depth profiles for the lakes under investigation. The graphs show that, in general, the lakes are either completely mixed or else there is a smooth, gradual drop in temperature from top to bottom. Thus the lakes approximate to the "3rd Order Temperate-type" of Whipple's

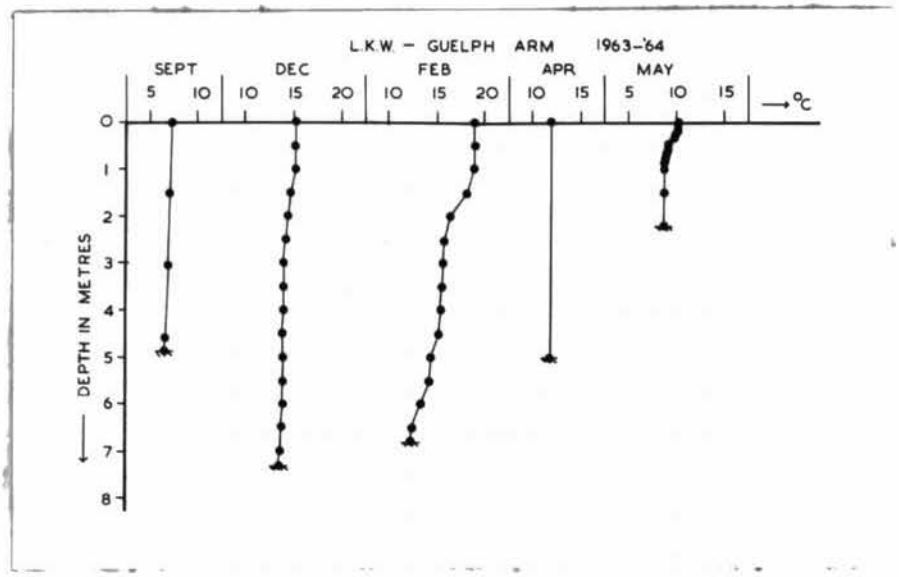


Fig. 4

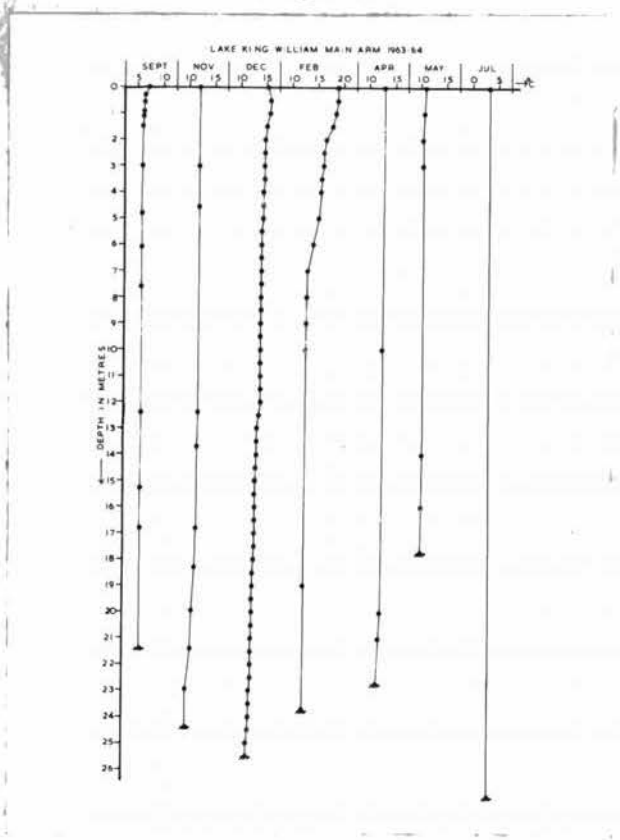


Fig. 5

Figs. 4-5. Temperature/depth profiles of the Guelph Arm and Main Arm of Lake King William. The hatched horizontal line represents the lake bottom.

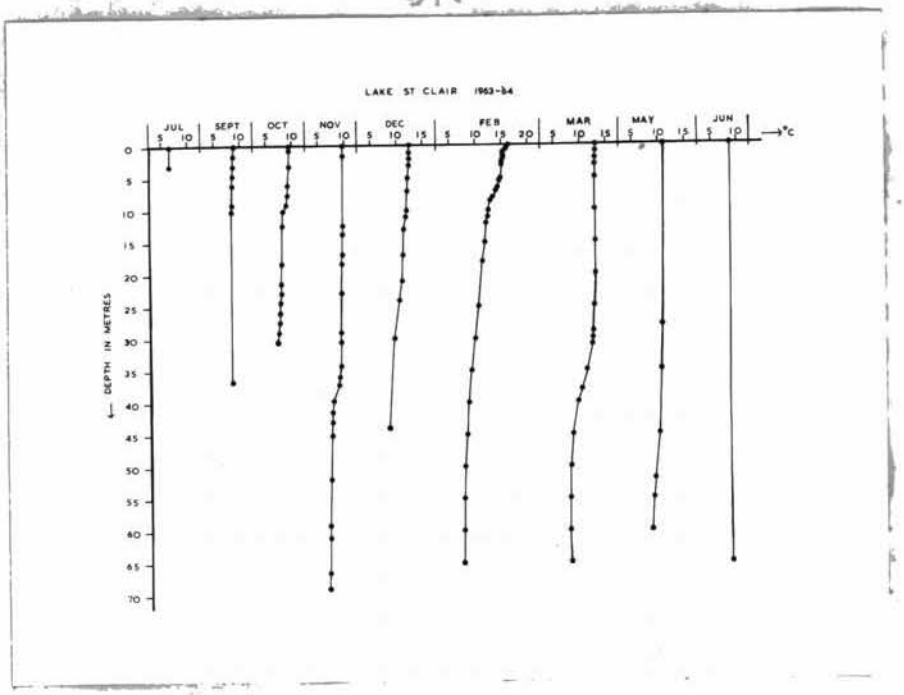


Fig. 6

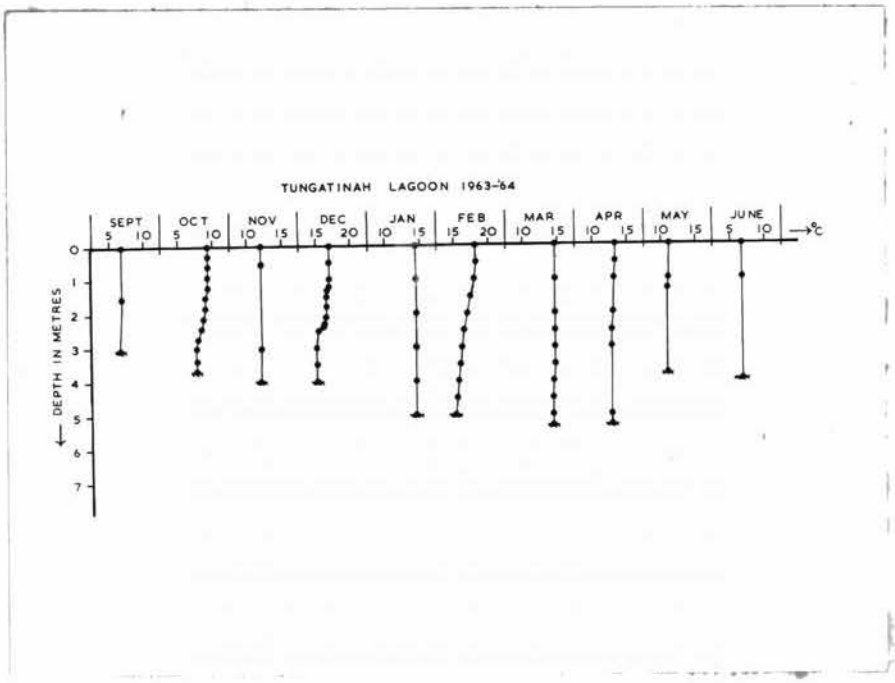


Fig. 7

Figs. 6-7. Temperature/depth profiles for Lake St. Clair and Tungatinah Lagoon. Temperatures were not recorded below 70m in Lake St. Clair.

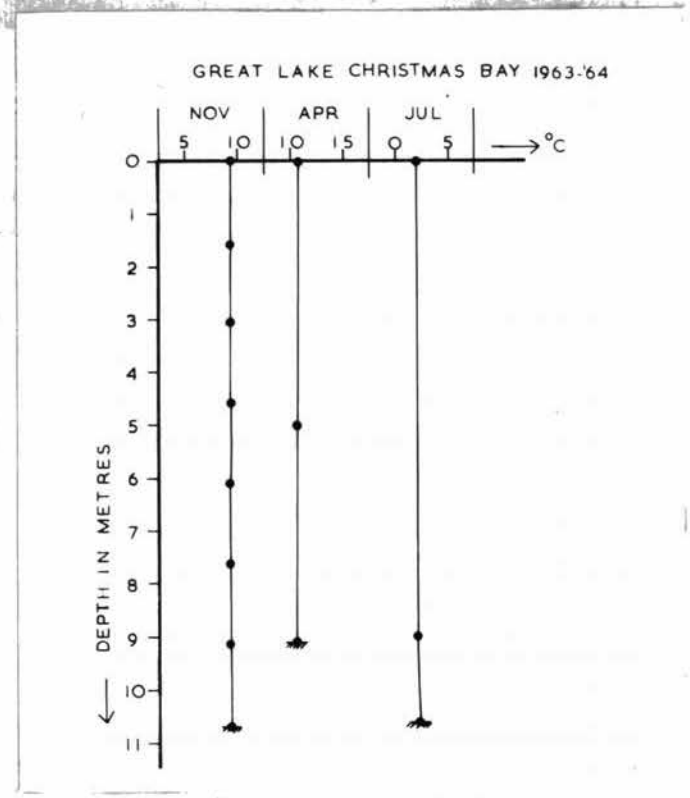


Fig. 8

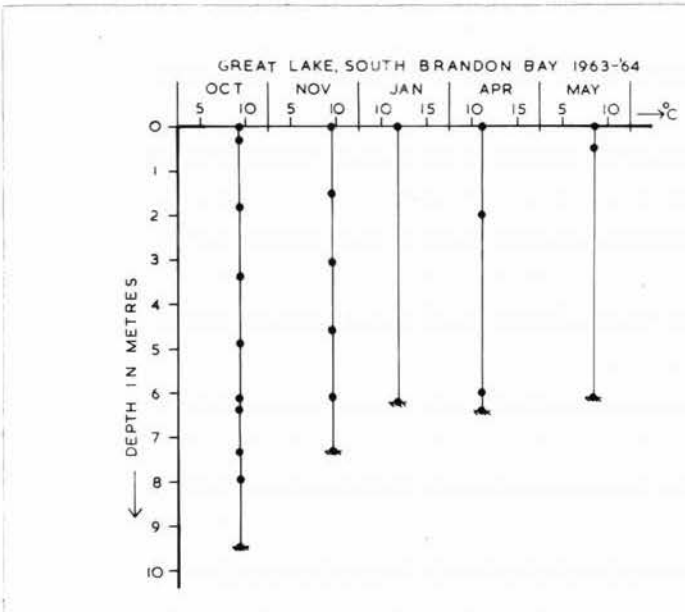


Fig. 9

Figs. 8-9. Temperature/depth profiles for the Christmas, and South Brandon Bays stations of Great Lake.

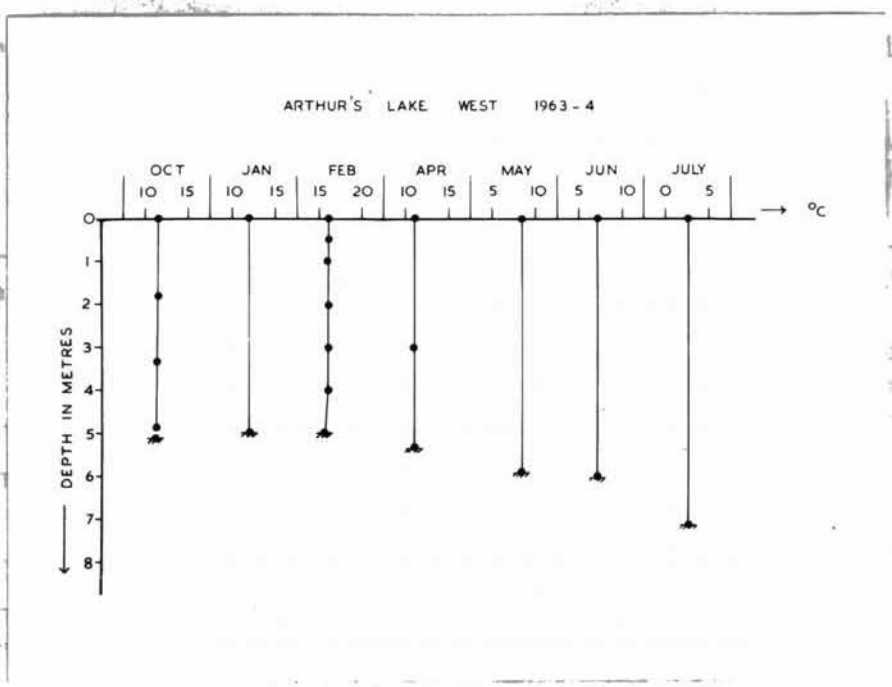


Fig. 10

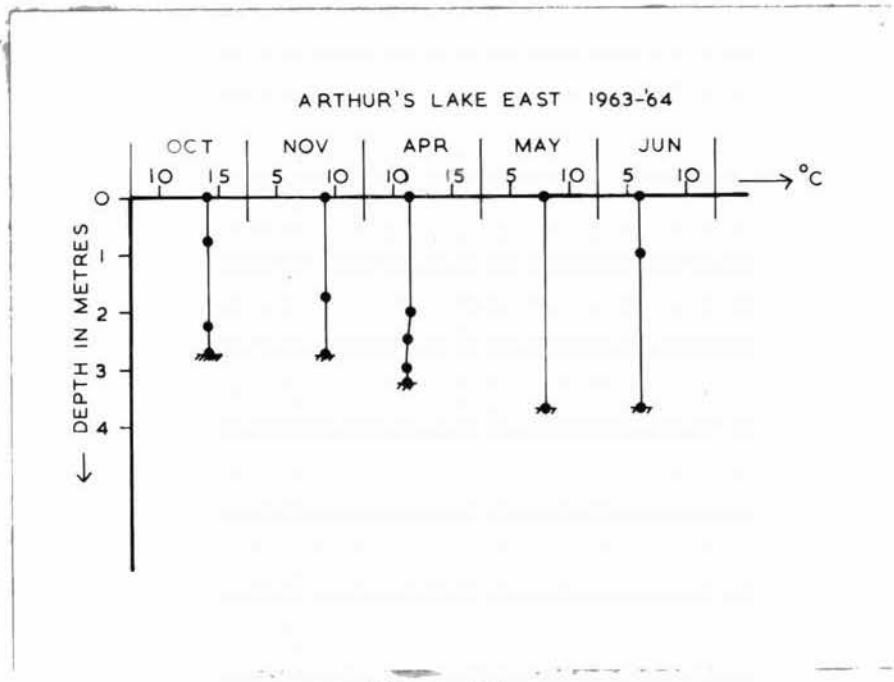


Fig. 11

Figs. 10-11. Temperature/depth profiles for Arthur's Lakes West and Arthur's Lakes East.

Table 1 - Oxygen saturation values for certain lakes of the Tasmanian Central Plateau after correction for altitude *

Lake	Date	Sample depth (m)	Dissolved oxygen (mg/l)	Surface Temp. °C	Altitude of Lake surface (metres)	% oxygen saturation
Lake King William Guelph Arm	3.9.63	0	10.6	7.2	714	98.6
Lake King William Main Arm	2.7.63	0.9	11.2	4.2	714	98.6
	3.9.63	0	11.1	7.4	714	104.6
	3.9.63	20	10.7	5.8	714	98.1
	8.10.63	0	10.2	11.8	714	106.3
Lake St. Clair	9.10.63	0	10.5	9.4	737	103.5
		30	10.5	7.2	737	98.0
		61	10.7	-	737	-
Great Lake, Christmas Bay	3.7.63	0	11.6	2.0	1030	96.1
	31.7.63	0	11.8	2.5	1030	99.4
Great Lake, South Brandon Bay	3.7.63	0.9	11.3	2.0	1030	93.8
	31.7.63	0	11.6	2.2	1030	97.2

* Calculated from the nomogram of Mortimer (1956)

classification (Welch, 1935). However, during periods of calm they may have temporary thermal stratification or the beginnings of this, as in the shallow Guelph Arm of Lake King William in February 1964 (Fig. 4) and even the deeper main arm of this lake (Fig. 5) on the same day. In Tungatinah Lagoon, which is shallow and comparatively sheltered, this tendency is more frequent (Fig. 7).

(b) Dissolved oxygen

Dissolved oxygen determinations were carried out during the early months of the investigation. The results are shown in Table 1, where the dissolved oxygen concentrations have been converted to percentage saturation and corrected for the altitudes of the lakes. The results indicate that oxygen was distributed throughout the depth of the lakes at near saturation level. As the initial chemical reactions of the Winkler method must be carried out in the boat immediately after sampling, and as the lakes were invariably rough, the deviations from 100% saturation are more likely to be experimental error than true variations. Throughout the period of the investigation, temperature readings showed that the lakes were well-mixed and, therefore, oxygen determinations were discontinued. It is very probable that the lakes are highly oxygenated throughout their depth and throughout the year.

(c) Chemical conditions

The results of chemical analyses of the waters over a twelve month period are shown in Tables 2-5. All analyses were carried out by the Tasmanian Government Analyst using methods recommended by APHA (1960). The results are similar to those published by Williams (1964).

At the very low levels of total dissolved solids (TDS) found in these lakes, analyses become difficult and time-consuming. The ionic balances of the analyses reported here, with few exceptions, are reasonable for such low concentrations and the analyses do give a sufficiently clear picture of the state of the waters. However, it is the view of the author and of the Tasmanian Government Analyst that special methods need to be developed, or existing ones refined to suit the special requirements of such soft waters. The analyses for manganese, in particular, were difficult and unsatisfactory. The inadequacies of existing methods for manganese in fresh-waters have been recognised elsewhere (Brownley, 1958; Morgan and Stumm, 1965).

The outstanding feature of the lakes considered is their very low nutrient status, with TDS always < 50 mg/l and alkalinity < 10 mg/l. In this respect, they rank as "extremely soft" waters (Brooks and Deevey, 1963) and

Table 2 - Chemical features of the Main and Guelph arms of Lake King William from September 1963 to November 1964

Date	Sampling Depth (m)	Total Dissolved Solids (mg/l)	Fixed Solids (mg/l)	Organic Carbon (mg/l)	pH	Alkalinity (mg/l $CaCO_3$)	Na^+ (mg/l)	K^+ (mg/l)	Ca^{++} (mg/l)	Mg^{++} (mg/l)	Cl^- (mg/l)	Colour (Hazen)	Turbidity (mg/l SiO_2)	Secchi Transparency (m)
GUELPH ARM														
1963														
Sept.	0				6.8	2.4	3.4	0.2	1.1		3.0			
Oct.	0				6.4	3.0	3.1	0.15	1.1	0.6	3.4		0.54	
Dec.	0	26.3	15.8	2.0	6.4	4.0	5.1	0.25	1.0	0.6				
1964														
Feb.	0	27.0	17.4	2.0	6.6	4.0	4.0	0.26	1.25	0.8	6.4			
Mar.	0	25.1	17.0	2.5	6.4	4.5	4.7	0.26	1.2	0.6	5.2	5	0.86	
Apr.	0	29.2	16.4		6.6	4.0	3.2	0.18	1.4	0.8	6.2	10	0.38	3.5
May	0	31.1	17.8		6.4	4.0	4.0	0.19	1.1	0.9	5.3	15	1.9	2.2
Nov.	0				6.7	3.2						10-15	0.75	4.1
MAIN ARM														
1963														
July	0				6.2	3.0	4.2	0.3			4.0			
	9.1				6.1	3.0	3.3	0.1			4.5			
Sept.	0				6.8		3.2	0.3	1.3		3.2			
	20				6.8	2.4	2.9	0.35	1.4		2.7			
Oct.	0				6.3	3.0	3.4	0.20	1.55	0.28	3.4		0.28	
	20				6.3	3.0	3.1	0.20	1.44	0.43	3.4		0.25	
Nov.	0				6.4	4.0								
Dec.	0	32.4	29.1	1.0	6.4	4.0	4.2	0.22	1.4	0.8	6.7			
1964														
Jan.	0	19.9	14.6	2.4	6.7	4.0	3.6	0.26	1.0	0.3	6.1			
Feb.	0	24.4	17.6	1.8	6.5	4.4	3.8	0.35	1.3	0.5	6.4			
Mar.	0	24.8	18.1	2.0	6.4	4.0	4.7	0.38	1.3	0.7		5	0.58	4.1
Apr.	0	25.7	17.5		6.7	3.0	3.2	0.26	1.1	0.5	6.4	10	0.25	5.0
May	0	25.2	16.8		6.4	4.0	3.7	0.27	1.4	0.8	5.8	<5	4.55	1.1
June	0	21.9	14.9	2.0	6.6	4.0	3.8	0.25	1.0	0.6	7.0	10	0.74	
July	0	25.0	15.2	1.5	6.4	4.2	3.4	0.32	1.1	0.7	5.0	<5	0.58	4.6
Nov.	0	24.0	16.0	1.5	6.6	3.2	3.2	0.19	1.0	0.35		5	0.50	6.0

Table 3 - Chemical features of Lake St. Clair from September 1963 to November 1964

Date	Sampling Depth (m)	Total Dissolved Solids (mg/l)	Fixed Solids (mg/l)	Organic Carbon (mg/l)	pH	Alkalinity (mg/l $CaCO_3$)	Na^+ (mg/l)	K^+ (mg/l)	Ca^{++} (mg/l)	Mg^{++} (mg/l)	Cl^- (mg/l)	Colour (Hazen)	Turbidity (mg/l SiO_2)	Secchi Transparency (m)
1963														
Sept.	0				7.0	2.4	2.6	0.2	1.3		2.0			
Oct.	0				6.5	3.5	2.6	0.2	1.13	0.53	2.5		0.2	
	61				6.5	3.5	2.8	0.2	1.34	0.41	2.7		0.2	
Nov.	0				6.8	4.0								
	59				6.6	4.0								
Dec.	0	22.8	15.4	1.6	6.4	3.8	2.4	0.21	1.0	0.5				
1964														
Jan.	0	22.5	13.5	1.3	6.8	3.5	3.4	0.21	1.0	0.5	3.9			
Feb.	0			1.0	6.2						6.1			
Mar.	0	18.1	13.1	1.2	6.4	4.0	3.5	0.26	1.0	0.4		<5	1.20	11.5
Apr.	0	23.9	16.5		6.8	4.0	3.2	0.27	1.3	0.5	5.2	5	0.40	10.5
May	0	26.2	18.3		6.6	3.8	3.7	0.27	1.1	0.6	5.8	<5	0.25	13.3
June	0	20.4	14.9	0.77	6.7	3.8	3.5	0.21	1.0	0.5	7.3	<5	0.14	10.7
Nov.	0	20.0	15.0	2.4	6.7	3.2	3.1	0.28	1.1	0.3		5	0.10	9.3

Table 4 - Chemical features of Christmas Bay and South Brandon Bay stations of Great Lake, from July 1963 to May 1964

Date	Sampling Depth (m)	Total Dissolved Solids (mg/l)	Fixed Solids (mg/l)	Organic Carbon (mg/l)	pH	Alkalinity (mg/l $CaCO_3$)	Na^+ (mg/l)	K^+ (mg/l)	Ca^{++} (mg/l)	Mg^{++} (mg/l)	Cl^- (mg/l)	Colour (Hazen)	Turbidity (mg/l SiO_2)	Secchi Transparency (m)
CHRISTMAS BAY														
1963														
July	0				6.8									
	0				6.5	4.0								
Sept.	0				7.2	3.9		0.2	1.2					
Oct.	0				6.8	4.5		0.2	1.0	0.5			0.22	
Nov.	0	23.0	15.0	1.6	7.1		3.2	0.22	1.0	0.3				
Dec.	0	32.6	29.3	1.1	6.5	4.0	3.8	0.34	2.9	0.5				
1964														
Jan.	0	15.0	10.7	1.2	6.8	4.5	2.8	0.23	1.0	0.1	4.2			
Feb.	0			1.0	o.9						6.1			
Mar.	0	20.2	15.5	0.9	6.5	4.5	3.3	0.32	1.2	0.6	3.2	<5	0.74	7.2
Apr.	0	20.6	14.2		6.8	4.0	2.2	0.22	0.8	0.4	4.3	<5	0.4	8.4
June	0	19.2	14.0	1.2	6.8	4.8	3.3	0.25	1.0	0.5	5.2	<5	0.25	
July	0	19.4	14.0	0.9	6.6	4.8	2.6	0.27	1.2	0.1	2.7	<5	0.14	5.4
Nov.	0				7.2	3.8						<5	0.75	
SOUTH BRANDON BAY														
1963														
July	0				6.9			0.2	1.4					
	0				6.4	3.0								
	6				6.5	4.0								
Oct.	0				6.7	4.0	1.6	0.2	0.9	0.14			0.14	
Nov.	0	18.0	17.0	0.8	7.0	4.0	2.9	0.2	0.9	0.2				
Dec.	0	18.3	18.2	1.0	6.7	5.0								
1964														
Jan.	0	13.8	10.0	0.6		4.5	2.7	0.2	0.9	0.2	5.2			
Mar.	0	17.4	12.2	1.0	6.5	4.3	3.0	0.28	1.1	0.2	3.4	<5	0.25	>7
April	0	19.2	13.6		7.0	4.0	2.3	0.22	0.8	0.4	3.9	<5	0.38	
May	0	22.2	16.1		6.7	4.0	3.8	0.28	0.9	0.6	4.2	<5	1.0	4.2

Table 5 - Chemical features of West and East sample stations of Arthur's Lakes, from July 1963 to November 1964

Date	Sampling Depth (m)	Total Dissolved Solids (mg/l)	Fixed Solids (mg/l)	Organic Carbon (mg/l)	pH	Alkalinity (mg/l $CaCO_3$)	Na^+ (mg/l)	K^+ (mg/l)	Ca^{++} (mg/l)	Mg^{++} (mg/l)	Cl^- (mg/l)	Colour (Hazen)	Turbidity (mg/l SiO_2)	Secchi Transparency (m)
ARTHUR'S LAKES (WEST)														
1963														
July	0				7.0		2.5	0.2	1.6					
Aug.	0				6.4	5.0	2.8							
Sept.	0				7.2	5.8	2.2	0.4	1.7					
Oct.	0				6.8	6.5	2.2	0.35	2.0				1.42	
Nov.	0	37.0	34.0	1.9	7.0	7.0	3.0	0.32	1.5	0.4				
Dec.	0	33.8	25.8	1.0	6.8	7.8	3.4	0.44	1.8	0.4				
1964														
Jan.	0	29.4	21.2	2.2	6.7	6.0	3.0	0.46	1.7	0.2	5.2			
Feb.	0.5	38.0	26.4	1.4			5.1	0.85	2.3	0.6				
Mar.	0	43.9	33.0	2.7	6.5	9.5	4.0	0.81	2.3	0.9	5.8	<5	0.58	2.4
Apr.	0	40.5	28.3		7.0	8.0	2.9	0.64	1.9	1.0	5.3	5	0.66	
May	0	44.3	30.0		7.0	10.0	4.1	0.86	2.8	0.4	5.5	10		2.9
June	0	38.5	28.0	2.6	7.1	10.0	4.0	0.59	2.0	0.7	6.4	10	0.74	2.2
July	0	36.6	23.2	1.9	6.8	10.0	3.6	0.67	2.3	0.7	4.7	10	0.51	2.7
Nov.	0				7.5	8.2						15	2.0	1.8
ARTHUR'S LAKES (EAST)														
1963														
Sept.	0		0			3.9	1.8	0.2	1.2					
Oct.	0		0		6.8	4.0	1.8	0.2	1.3	0.20			1.8	
Nov.	0				6.9	5.0								
Dec.	0	22.2	17.2	1.4	6.7	3.8	2.8	0.26	0.8	0.3	4.9			
1964														
Jan.	0	27.8	21.5	1.7	6.8	4.5	3.4	0.34	1.2	0.1	4.9			
Feb.	0	31.8	24.4	0.9	6.4	4.8	3.4	0.38	1.2	0.6	5.6			
Mar.	0	31.6	23.6	2.0	6.5	5.0	3.9	0.39	1.8	1.0	3.4	5	5.7	1.15
Apr.	0	34.1	25.5		6.8	5.0	2.6	0.31	1.0	0.5	4.9	5	15.0	0.8
May	0	35.8	27.4		6.8	4.8	3.0	0.34	1.0	0.1	4.2	<5	13.4	1.1
June	0	30.6	24.2	0.7	6.9	4.8	3.4	0.35	1.1	0.5	4.6	<5	7.5	0.9
Nov.	0	42.0	30.0	1.2			3.7	0.40	1.6	0.7				

compare with alpine lakes in other parts of the world (Williams, 1964). The intrinsic colour of Lake King William is usually 5-10 Hazen° in contrast to the clear, colourless waters of Great Lake and Lake St. Clair. There is relatively little variation in the concentration of the various ions through the year and the pH is constant and slightly acid. The TDS of Great Lake and Lake St. Clair are somewhat lower than those of Lake King William but the amount of total fixed solids is comparable in all these lakes. This fact, plus the generally higher organic carbon content of Lake King William confirms the presence in this lake of relatively large amounts of organic matter which the brown colour of the water suggests. Arthur's Lakes are little different from the other lakes considered though there is a tendency for increased TDS values, particularly in Arthur's Lakes West. High turbidity and low transparency are characteristic of both Arthur's lakes but more particularly the Eastern one.

(d) Phytoplankton

Tasmanian freshwater algae have never been investigated and there are no publications dealing with the plankton. Consequently, any investigation of Tasmanian freshwater ecology must commence with taxonomic determinations. Early in this investigation it was realized that detailed

knowledge of the plankton would have limited application to this problem and investigation was limited to a qualitative comparison of the lakes.

At this level, there is little difference between any of the lakes. All appear to be dominated by a desmid flora rich in such genera as Cosmarium, Staurastrum, Arthrodesmus, Triploceras and Xanthidium, and a mixed population of colonial Chlorococcales. In addition, Dinobryon is a constant member of the plankton. With the exception of a sudden crop of Rhizoslenia in Arthur's Lakes East during June 1964, the plankton of all lakes is poor in diatoms. There is a decided tendency for Great Lake to be poorer in species and in numbers and for Arthur's Lakes East to contain the greatest variety of species and the densest populations.

2. Analysis of pipeline deposits

(a) Description of deposits

In the pipelines of the Derwent system, carrying the waters of Lake King William, the deposit builds up to a maximum thickness of about 7 mm. It takes approximately 6 months to reach this peak. It occurs as a soft wet deposit whose surface consists of a series of ridges and

folds, giving a rippled appearance (Fig. 12). This presumably is produced by flow patterns in the water, as ripples on a sand beach, since the rippling consists of a regular, repeating pattern of troughs and crests, with approximately constant wavelength (Fig. 12). Periodic patterns in deposited materials in pipelines has been studied by Thomas (1964). The photographs published by Schweisfurth and Mertes (1962) show that a ripple pattern is present on the surface of deposits in pipelines at Trier, Germany. The deposit is easily scraped from the pipe surface, then appearing as an amorphous, dark black-brown mass. If rubbed between the fingers it stains them purplish-brown and leaves a distinctive odour. If the deposit is allowed to dry on the pipe surface it does so as a smooth, hard, enamel-like surface. If a sample is dried in air or in the oven it forms a crumbly, dark black powder.

In contrast to this, deposits in the Shannon and Waddamana pipelines are extremely thin, even after long periods of continuous operation (Fig. 13). In addition they are entirely different in appearance from the Derwent deposits. They occur as a soft, grey-brown lining to the pipelines and often contain the tubular tunnels of Chironomid larvae (Fig. 13).

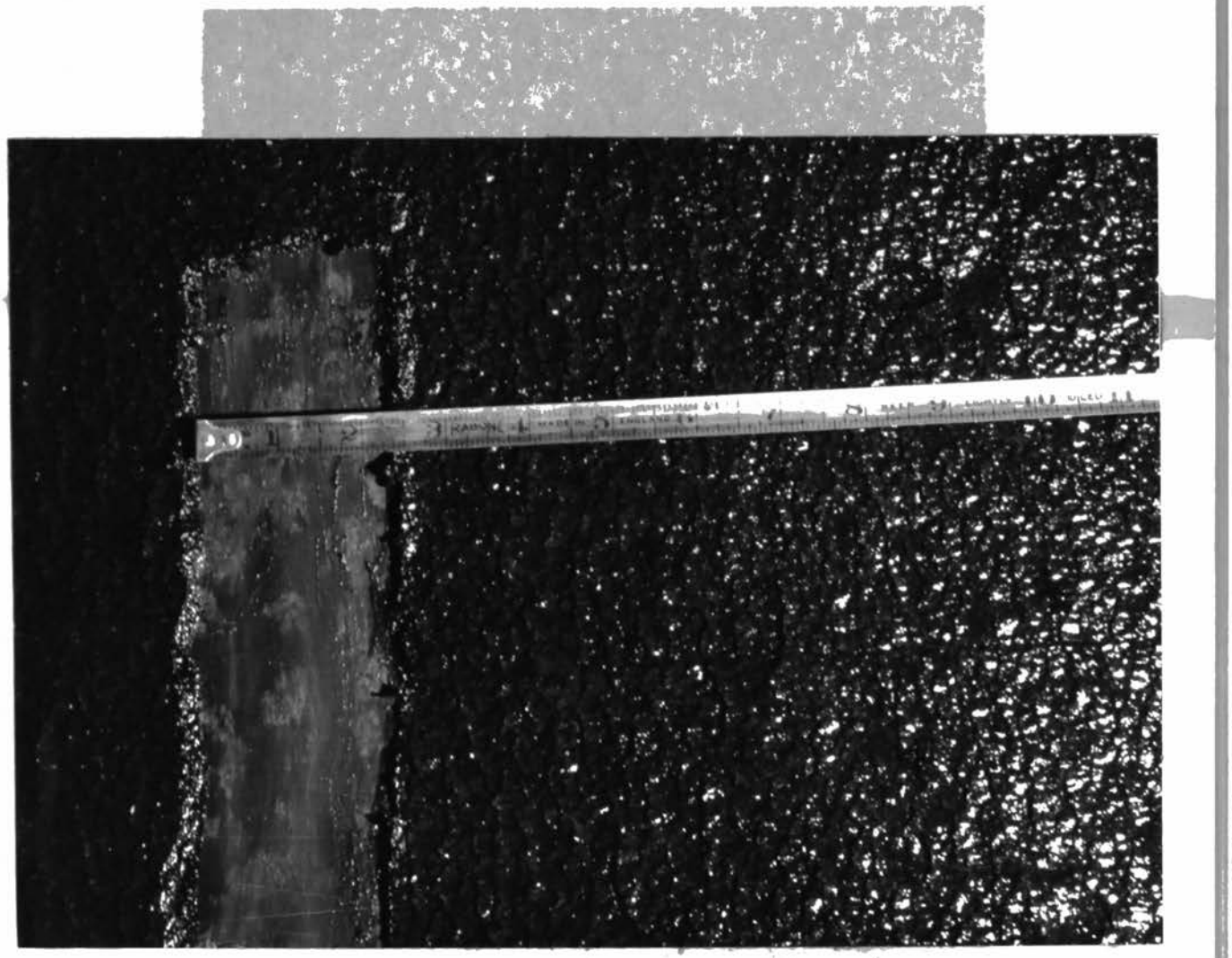


Fig. 12. Manganese deposits in Derwent pipelines, Tasmania, showing regular ripple pattern. The shadow at the right hand edge of the scraped portion of pipe gives an indication of the thickness of the deposit.

Several deposits from other parts of Australia and elsewhere have been examined, including those from the pipelines, North Queensland, [illegible] to that of the Snowy Mountains and the [illegible] mediate in [illegible] especially

Fig. 13. Deposits in the Waddamana pipelines, Tasmania, showing thin, light-coloured nature. The tubular objects are the burrows of larvae of the Chironomidae (Diptera).

Table 6 - Analyses of pipeline deposits from Tasmania and other parts of Australia.
All analyses expressed as a percentage of the oven dry weight of deposit.

Location	Loss on ignition at 550°C	SiO_2	Fe	Fe+Al (as Fe)	Al	Mn	Ca	Mg	Appearance	Microorganisms present
Waddamana) Great Lake	31.1	25.0	-	25.5	-	1.9	0.6	0.2)	Light-brown	-
Shannon) Tasmania	54.2	7.4	-	19.4	-	00.6	-	-)		-
Tarraleah)	24.0	4.5	-	8.5	-	34.0	2.8	0.5)		
")	30.9	6.2	4.0	-	2.5	28.6	-	-)		
")	21.6	5.6	-	13.5	-	36.2	-	-)		
")	20.8	8.5	5.3	-	2.7	30.7	3.6	0.7)		
")	23.4	9.8	5.5	-	4.0	32.5	1.4	0.3)		
")	19.0	9.0	5.8	-	6.1	23.7	3.8	2.2)		Hyphomicrobium Dominant.
")	24.0	4.5	-	8.4	-	33.9	2.8	0.6)		
")	28.2	7.5	4.1	-	2.7	31.8	-	-)		Chlamydobacteria scarce.
") Derwent	19.6	2.1	6.8	-	0.8	37.4	3.4	0.1)		
") pipelines,	19.9	3.7	-	7.9	-	33.3	2.9	1.1)	Dark brown-	Some cocci and
") Tasmania	16.9	4.8	-	9.2	-	33.9	2.6	1.3)	black	bacilli.
")	17.2	3.9	-	7.2	-	34.2	2.8	2.0)		
")	17.2	3.0	-	8.1	-	36.5	2.0	1.4)		
Liapootah)	21.9	8.3	-	9.9	-	31.0	3.2	0.3)		
")	26.2	13.7	-	18.3	-	12.1	2.7	3.1)		
")	22.5	8.2	-	10.5	-	27.0	2.1	1.0)		
")	21.9	8.3	-	9.9	-	31.0	3.2	0.3)		
")	20.9	8.7	-	8.7	-	20.6	2.3	0.7)		
Wayatinah)	32.5	11.9	5.2	-	3.9	23.7	-	-)		
Catagunya)	24.7	13.9	-	13.8	-	20.0	3.4	0.5)		
Marrawah irrigation	23.1	18.1	-	20.6	-	11.8	1.0	0.4	Dark brown	Bacilli and cocci.
Kareeya	19.0	2.9	11.3	-	0.7	33.8	1.1	0.1	Dark brown-black	Hyphomicrobium
Tumut No.1, Snowy Mountains	24.8	16.6	7.4	-	3.8	18.6	2.7	0.1	Dark brown	Hyphomicrobium and Chlamydobacteria.
Eppalock-Bendigo Victoria	11.0	23.3	10.0	-	3.3	19.8	3.3	0.6	Dark brown	Bacilli and cocci. Few hyphomicrobia.

The similarities in composition between Derwent deposits and those from many other parts of the world is striking. For this reason analytical data from several parts of the world is presented in Table 7 for comparison. Again, variations in iron and silica content are to be expected, depending on the nature of the surface from which the sample was taken. Published results have been included only where it was clear that the deposit was taken from the wall of a pipeline or tunnel, where the only contaminants would be rust and silica.

3. Laboratory investigation of manganese deposition from natural waters

(a) A recirculatory apparatus

To overcome the problem of access to pipelines at regular intervals, a recirculatory apparatus (Fig. 3) was devised, based on the perfusion methods used in studies of mineral cycles in soils. A sample of 200 litres of water from Lake King William was circulated in the apparatus and, after 24 hours, a brownish deposit built up on the cover-slips in the tube (Fig. 14) and also wherever flow was interrupted, such as at the junction of glass and plastic tubes. This deposit built up progressively over 6 days, after which time no deposition took place on

Table 7 - Analyses of pipeline deposits from various parts of the world, for comparison with Australian figures. All analyses expressed as a % of the oven dry weight of the deposit and in the form stated. Conversion has been carried out where necessary.

Location	Loss on ignition	SiO_2	Fe	Fe+Al (as Fe)	Al	Mn	Ca	Mg	Reference
Trier, Germany	19.5	22.4	8.7	-	-	22.6	-	-	Schweisfurth and Mertes, 1962
" "	13.6	-	5.9	-	-	50.0			
" "	18.5	14.2	2.8	-	1.4	34.4	0.1	0.2	
" "	21.9	11.3	1.0	-	3.2	25.1	0.6	-	
" "	24.6	7.2	1.2	-	2.7	26.8	0.3	-	
Brooklime, Mass., U.S.A.	17.9	5.0	14.3	-	0.7	35.8	3.0	-	Weston, in von Wolzogen-Kuhr, 1927
Newton, Mass., U.S.A.	11.9	7.3	-	8.9	-	48.0	-	-	
" " "	27.9	12.5	12.6	-	0.8	21.5	-	-	Jackson, 1902
Brainerd, Minn., U.S.A.	18.0	16.2	13.3	-	0.2	29.0	1.3	1.8	Zapffe, 1931
" " "	23.5	7.1	14.5	-	0.4	29.9	4.0	0.3	
" " "	22.4	5.5	18.5	-	-	29.1	2.0	0.2	
Unstated, U.K.	27.7	-	24.9	-	-	25.8	-	-	Brown, 1904
Sheffield, U.K.	21.1	8.8	-	10.8	-	27.0	6.4	0.7	Waterton, 1954
Manchester, U.K.	14.4	8.7	-	2.2	-	28.4	2.5	-	
Gloucester, U.K.	21.7	6.4	-	19.6	-	22.0	3.6	0.8	

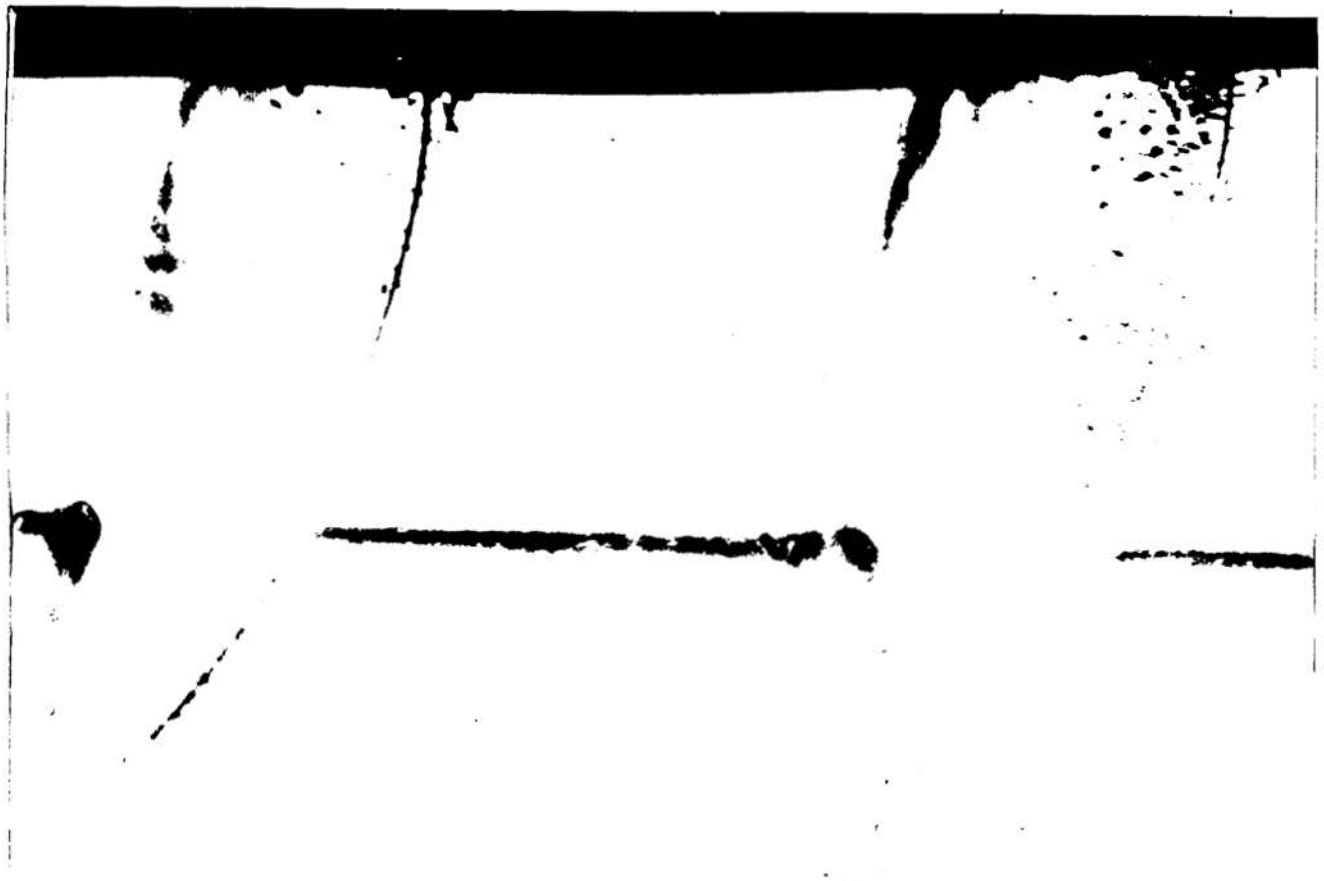


Fig. 14. Manganese deposits building-up on coverslips in a tube of the recirculatory apparatus, using Lake King William water.

fresh coverslips. If sterile manganous sulphate was added, deposition continued. The presence of manganese was confirmed by the benzidine test and by oxidation to permanganate with periodate.

Flowing water was essential for deposition to take place. When water was passed across the diameter of a tube instead of along the length of it, a deposit formed on only those coverslips in the flowstream.

(b) Deposits from Lake King William and Great Lake waters

Samples were taken from Lake King William and from Great Lake on the same day and recirculated in separate units of the laboratory apparatus. After five days a relatively heavy deposit has built up on coverslips in the Lake King William unit, while in the Great Lake unit only a very slight deposit had developed (Fig. 15). Thus the laboratory system reproduced precisely the field experience. This was confirmed by further comparative tests in the same manner.

(c) The involvement of microorganisms in deposition of manganese

Using Lake King William water three sterile units of the recirculatory apparatus were set up, each containing

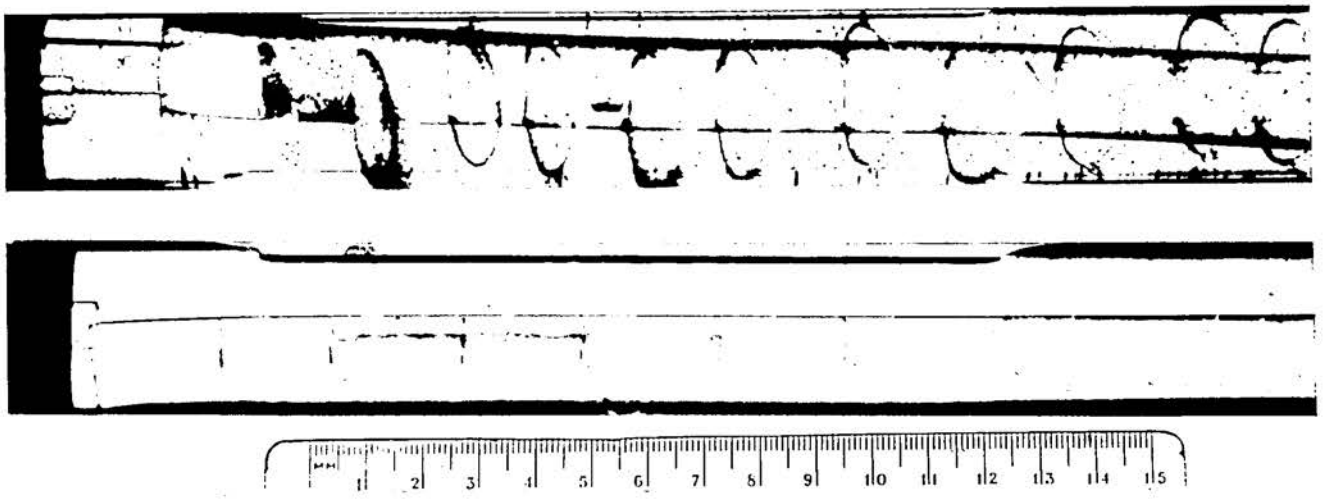


Fig. 15. Tubes from the recirculatory apparatus after 5 days circulation, showing considerable build up of manganese deposit in the Lake King William unit (above) in contrast to slight deposition in the Great Lake unit (below).

200 litres of the water treated as follows:

A - Untreated

B - Autoclaved at 120°C for 1 hour

C - With addition 10^{-3}M sodium azide as metabolic inhibitor.

After a few days, deposit had built up in Unit A only. An inoculum of 100 mls of Lake King William water was then added to Units B and C and the water circulated for a further period. A deposit was produced in the unit containing autoclaved water but in unit C the metabolic inhibitor continued to suppress growth of bacteria and, therefore, oxidation of manganese (Table 8). These results show that microorganisms are essential at least to initiate deposition of manganese.

(d) <u>The lack of available manganese in Great Lake water</u>

Three sterile units of the recirculatory apparatus were set up, each containing 200 litres of Great Lake water. Sterile manganous sulphate was added to one unit while 1 litre of Lake King William water was added to another to provide a source of manganese-oxidizing microorganisms. The third unit was the untreated control. After five days of circulation a heavy deposit of manganese had built up in the unit to which manganous sulphate was added while

in the other two units only a slight trace had developed. This demonstrates that the reason for the absence of deposition in Great Lake pipelines is not absence of the

Table 8. Demonstration of the involvement of microorganisms in manganese deposition from Lake King William water

Treatment	Relative amount of manganese deposition	
	Before inoculation	After inoculation
Control	+++	+++
10^{-3}M NaN_3	-	-
Autoclaved	-	++

in the other two units only a slight trace had developed. This demonstrates that the reason for the absence of deposits in Great Lake pipelines is not absence of the necessary organisms but lack of soluble manganese.

(e) Periodic variation in amount of manganese deposition

Lake King William and Great Lake were sampled at various times of the year and tested in the recirculatory apparatus. Results showed that there was variation in the amount of deposit produced from a standard volume of water from either lake. The results are shown in Table 9. Subjective ratings of "trace", "slight", "moderate" and "heavy" are used to denote the amount of deposit produced in the apparatus. At certain times of the year the amount of deposit produced from Lake King William is no more than slight. On occasions the Great Lake water also produced a slight deposit. The variation in amount of deposit does not fit into a seasonal pattern.

The figures for manganese concentration (Table 9) in the respective waters suggest that manganese levels in Great Lake are even lower than the low values recorded for Lake King William, supporting the evidence gained from addition of manganese sulphate to Great Lake water. However, in view of the unsatisfactory nature of available

Table 9. Periodic variation in the amount of manganese deposit produced in the test apparatus from a standard volume of Lake King William or Great Lake waters.

Lake	Date of Sample	Manganese concentration of water (mg/l)	Amount of deposit produced
King William	3/5/1964	0.071	Heavy
" "	5/6/1964	0.036	Heavy
Great Lake	4/6/1964	0.008	Trace
" "	28/7/1964	0.007	Trace
King William	8/9/1964	-	Slight
Great Lake	10/9/1964	-	Slight
King William	22/9/1964	-	Moderate
Great Lake	3/11/1964	0.013	Slight
King William	23/11/1964	-	Slight
King William	7/10/1965	0.010	Slight

analytical methods these tests were discontinued and much more investigation is needed.

(f) Effect of temperature on rate of manganese deposition

As the temperatures of Lake King William are relatively low throughout the year it was considered possible that the manganese-oxidizing bacteria were psychrophilic. To test this, two units of the apparatus were set up in constant temperatures of $4^{\circ}C$ and $25^{\circ}C$ respectively. Lake King William water was circulated in both units. The deposition commenced sooner in the unit at $25^{\circ}C$ and deposition continued at a faster rate in that unit, showing that the organisms which were capable of manganese oxidation are probably mesophilic and not psychrophilic.

(g) The probability of manganese deposition in future installations

For more than 40 years the water of Great Lake was led southwards to develop power in the Shannon and Waddamana power stations. Recently, the water has been deployed northwards through a new power station (Poatina) where a greater head can be obtained. As part of this scheme it was proposed to pump water from Arthur's Lakes across the watershed into Great Lake (Fig. 2). If Arthur's Lakes

ater produced a manganese deposit it would be troublesome n two ways. First, deposits in the pipeline between rthur's Lakes and Great Lake would result in increased umping costs and a reduction in power output from the mall powerstation at the point of entry into Great Lake. econdly, when the water mixed with Great Lake water it ould produce a deposit in the Poatina pipelines.

To test the probability of this happening, samples f water from Arthur's Lakes were taken on several occasions nd tested in the apparatus. On each occasion a moderate o heavy deposit developed. Arthur's Lakes water was ixed with Great Lake water in the proportion 1:3, the ikely ratio of the two waters when Arthur's Lake is being sed to maximum extent. In this case a moderate deposit eveloped. These results suggest that trouble could be xperienced with the Arthur's Lakes pumping scheme. As his only recently commenced operation it is too early to now whether a deposit has developed.

Planned future hydro-electric development now being onstructed in the north of Tasmania will utilize waters f the Mersey, Fisher, Forth and Wilmot Rivers. Tests ith these waters showed that only slight deposits were roduced, though addition of sterile manganese sulphate aused heavy deposits to develop in each case. However, manganese problem may develop once the waters are

impounded and these results cannot be used to confidently predict freedom from manganese problems in the new systems.

4. Microscopical examination of deposits

(a) Tasmanian deposits

Microscopical examination of fresh deposit from the Derwent pipelines is baffling. The deposit, which looks black when in bulk, appears golden-brown when viewed by transmitted light on the microscope slide. It appears amorphous and contains, and is surrounded by, a mixed population of bacilli, cocci and spirilla. These types of bacteria are readily recognized in any preparation. Sand grains, diatom frustules, the semicells of decayed desmids, loricae of Dinobryon (Chrysophyceae) and the remains of Cladocera and copepods are frequently entangled in the deposit. Where a piece of deposit has been torn by the mounting process, slender stalks may be seen bridging the tear (Fig. 16). These stalks are just within the limit of resolution of the optical microscope and careful examination by phase contrast is necessary to reveal them. Chlamydobacteria and fungal hyphae are rare.

If the manganese is dissolved away with 5% oxalic acid the true microbiological picture becomes clear. After dissolution of the manganese a colourless, amorphous

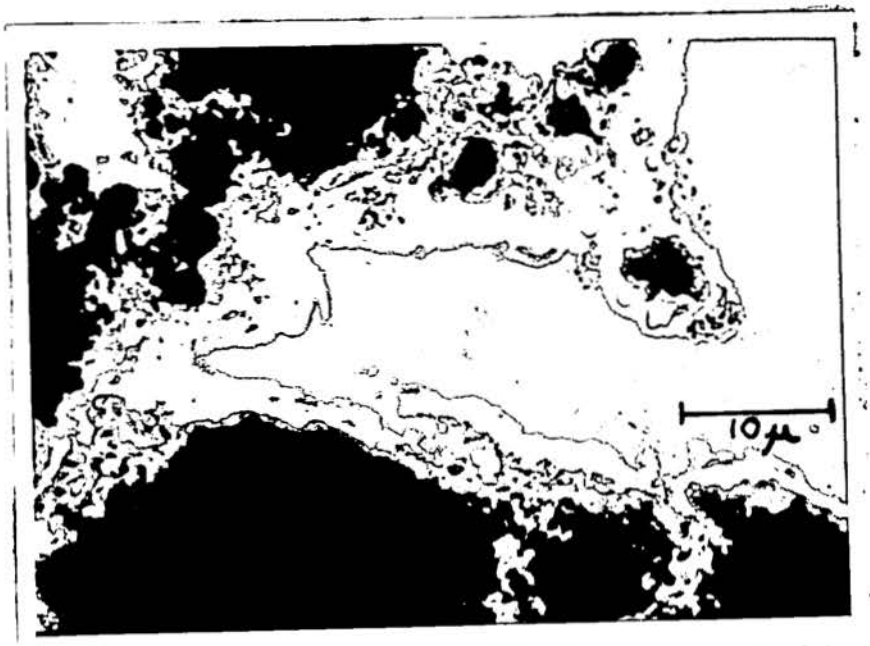


Fig. 16. Phase contrast microscopy of fresh deposit from the Derwent pipelines, showing slender stalks bridging a tear in the deposit.

material is left in which the presumed causative bacteria can be observed. Staining with carbol fuchsin facilitates observation. Under these conditions it can be seen that stalked, budding bacteria resembling Hyphomicrobium overwhelmingly dominate the Tasmanian deposit. The cells and branching stalks ramify as a close network throughout the deposit (Figs. 17, 18). Compared with the hyphomicrobia, all other types of bacteria are rare. Cocci and bacilli occur usually in isolated patches and they are not generally distributed throughout the deposit. Chlamydobacteria are rare and where they do occur they are always accompanied by a far greater number of hyphomicrobia (Fig. 19). Fungal hyphae are rarely seen in the deposit.

(b) Deposits from other parts of the world

Samples of manganese deposits were obtained from the Kareeya pipelines near Cairns, Queensland and from the Tumut pressure tunnel, Snowy Mountains Hydro-Electricity Authority, New South Wales. The Kareeya deposits were heavily dominated by stalked bacteria forming a network of cells and branching stalks ramifying through the deposit (Fig. 20). Chlamydobacteria are present but are far outnumbered by hyphomicrobia (Fig. 21). In the deposits from the Snowy Mountains, chlamydobacteria are more numerous and probably outnumber the hyphomicrobia (Fig. 22).

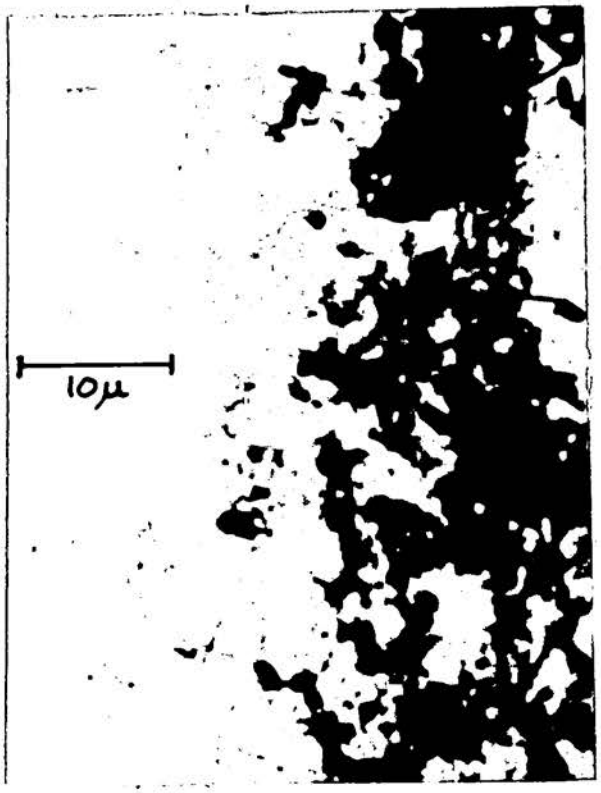


Fig. 17

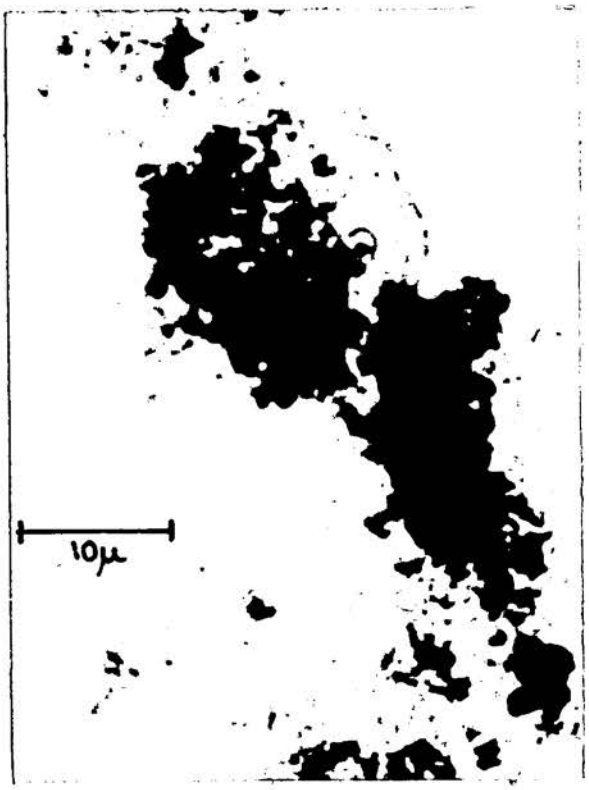


Fig. 18

Fig. 19

Figs. 17-19. Deposits from the Derwent pipelines of Tasmania, after removing manganese oxides with 5% oxalic acid and staining with carbol fuchsin. Figs.17, 18 show the network of Hyphomicrobium stalks and cells ramifying through the deposit. Fig. 19 shows chlamydobacteria and Hyphomicrobium.

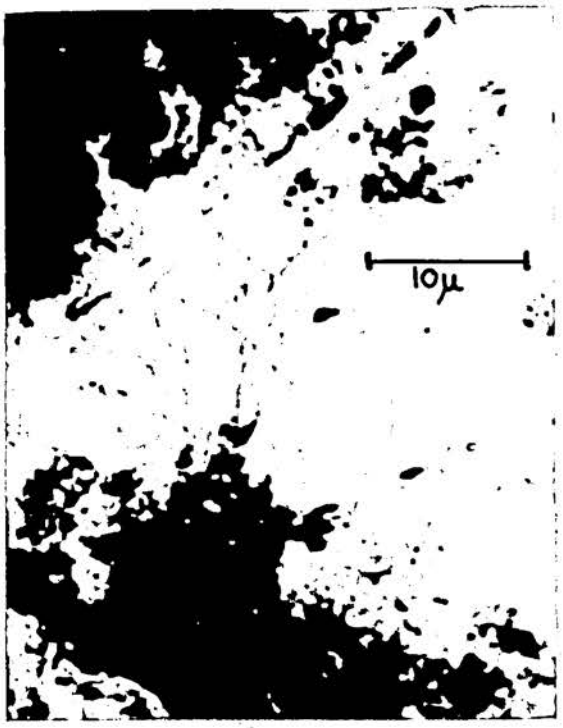


Fig. 20

Fig. 21

Figs. 20-21. Deposits from the Kareeya pipelines, near Cairns, Queensland, treated as in Figs. 17-19, showing hyphomicrobial network (Fig. 20) and occasional chlamydobacteria among the stalks (Fig. 21).

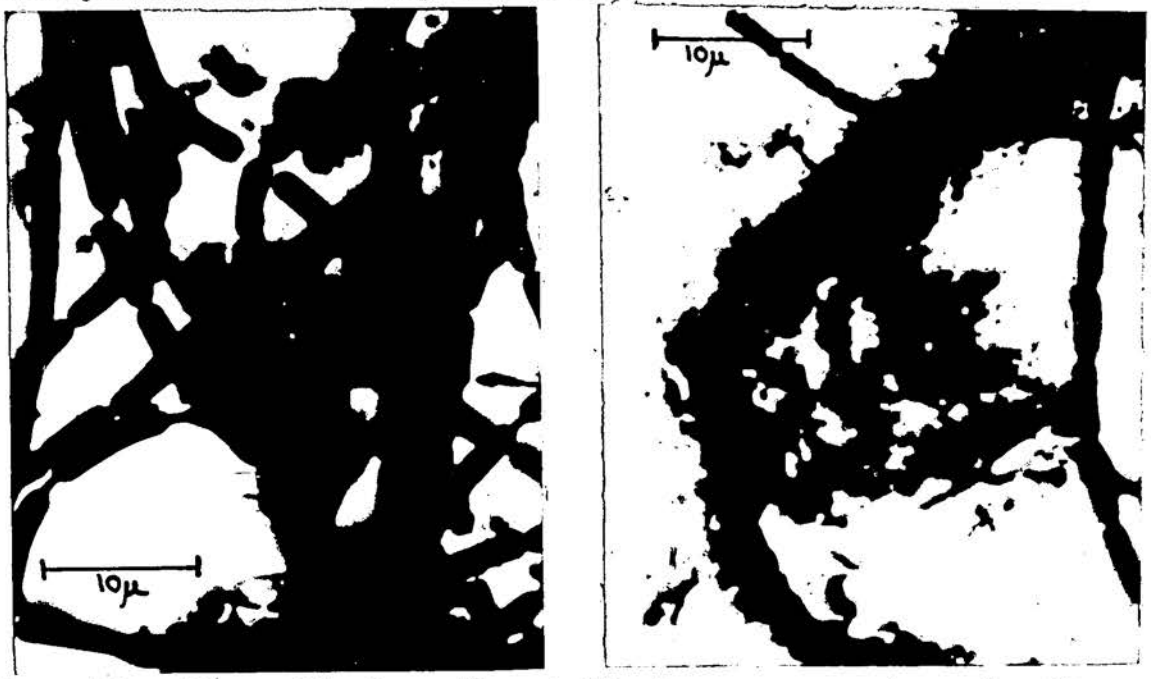


Fig. 22. Deposit from Tumut No.1 pressure tunnel, Snowy Mountains hydro-electric scheme, New South Wales, treated as for Figs. 17-19, showing numerous chlamydobacteria and few hyphomicrobia (left) and chlamydobacteria with an investment of hyphomicrobia.

(c) Deposits from the laboratory apparatus

When tests on various lake waters were conducted in the recirculatory apparatus the deposits which formed on the coverslips were examined. By wiping one surface of the coverslip the deposits on the other side could be examined *in situ* without disturbing the deposit.

Under the test conditions very many bacterial types were able to colonize the coverslip surface. However, wherever there was manganese deposit, stalked bacteria were also present. At the commencement of deposition it could be seen that the manganese oxides were first deposited around the cell part of the hyphomicrobia, leaving the stalks uncrusted (Fig. 23).

5. Isolation of manganese-oxidizing microorganisms

Small samples of fresh pipeline deposit were ground between two pieces of sterile ground-glass, suspended in sterile water and plated out in serial dilution on media PC and BM. Replicate plates were poured, incorporating Actidione in the medium to prevent growth of fungi which, though present in low numbers, swamped the plates before any bacteria had grown. Colonies of microorganisms which oxidized manganese were recognized by the brown colour of the deposited oxides. The following manganese-oxidizing

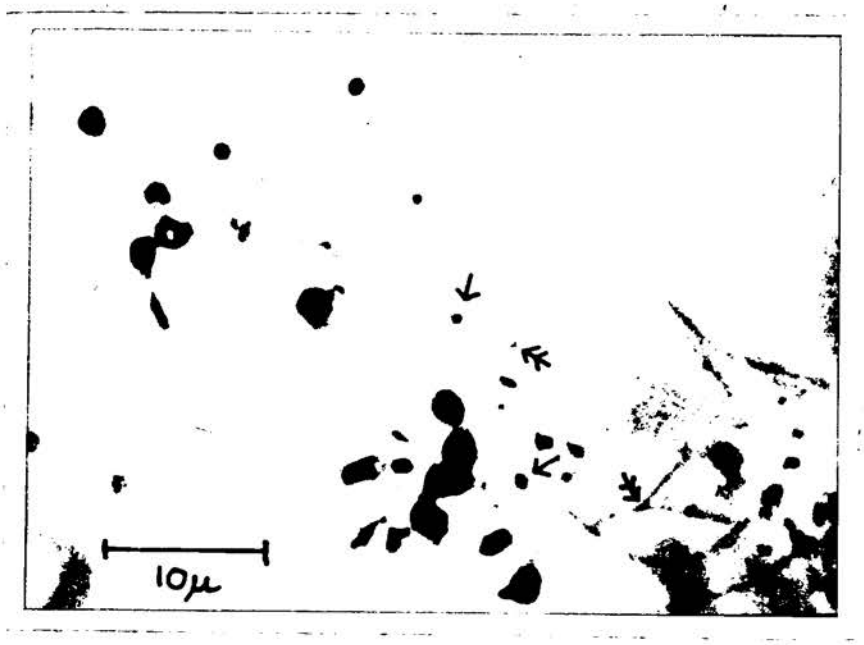


Fig. 23. An early stage in the build up of manganese deposits in the recirculatory apparatus, showing associated hyphomicrobia. Most cells have a thick coating of oxidized manganese. Note branching of stalks (double arrows), and cells not yet encrusted with manganese (single arrows).

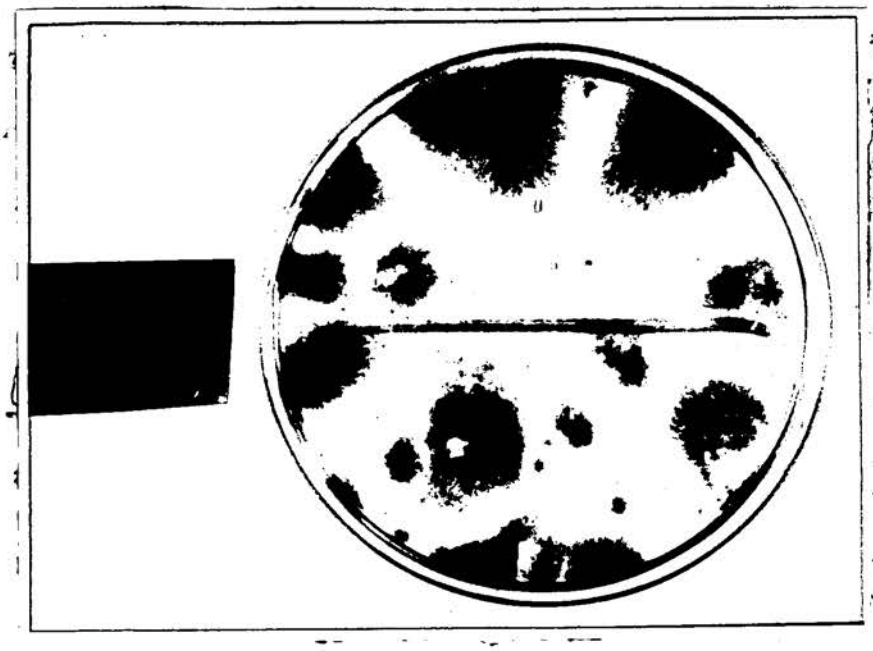


Fig. 24. Coniothyrium fuckelii Sacc., isolated from Derwent pipelines, oxidizing manganese on medium BM.

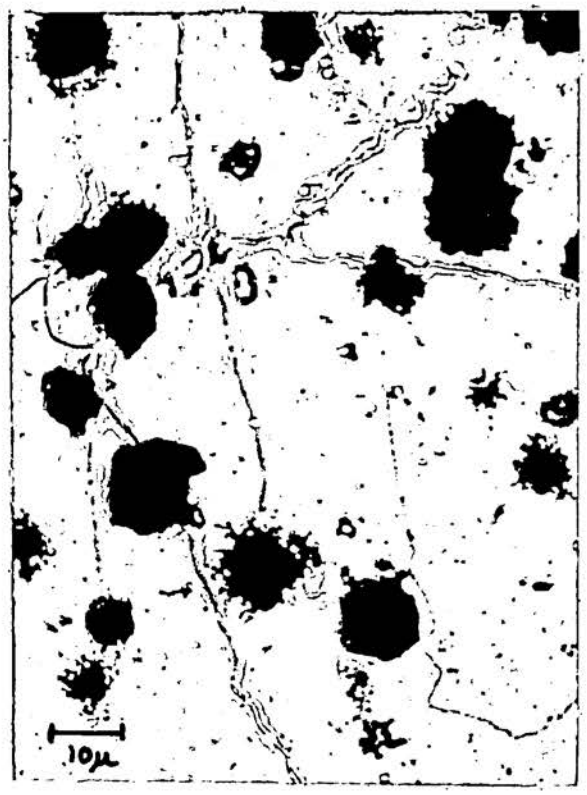


Fig. 25a

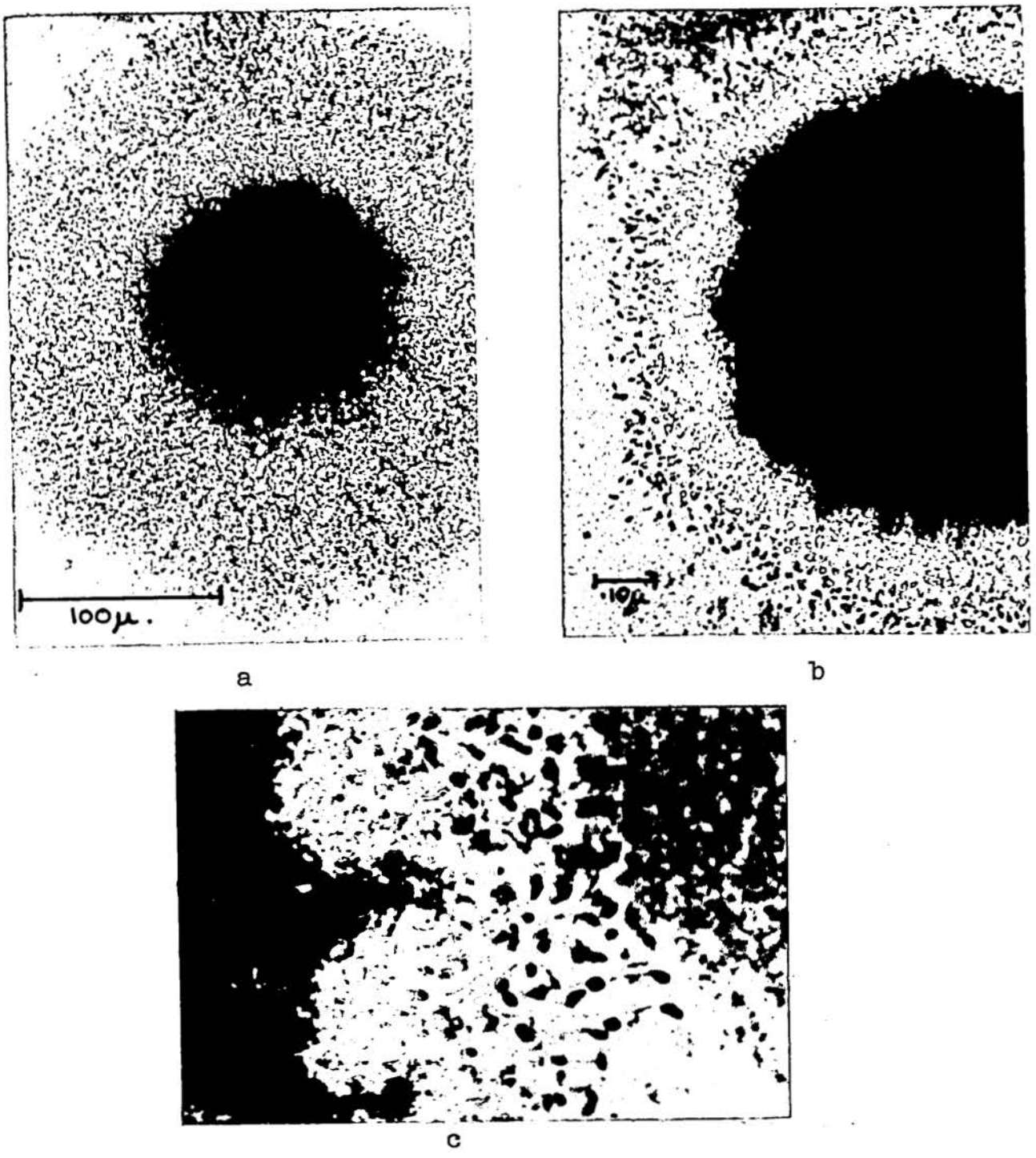
Fig. 25b

Fig. 25. Microcolonies of manganese oxidation in proximity to fungal hyphae (a), showing (b) the radiating, tapering threads described by Zavarzin (1961a) as Metallogenium symbioticum.

oxidized manganese vigorously in aerated liquid culture with PC medium. This bacterium was only isolated occasionally and, even then, in low numbers. For this reason and because rod-shaped bacteria are not common throughout the pipeline deposits, it was concluded that this organism is not an important one for the problem.

c) A stalked, budding bacterium resembling Hyphomicrobium. It formed colonies with dense brown-black centres of oxidized manganese. At the edge of the colony was a paler halo where the Hyphomicrobium cells and stalks projected beyond the zone of oxidation. The extent of this zone of oxidation varied from a small area in the centre, with a wide halo (Fig. 26a), to almost complete deposition, with cells visible only at the very edge (Fig. 26b) from which stalks of Hyphomicrobium projected radially into the medium, bearing unencrusted buds at the ends (Fig. 26c).

At the edge of many colonies the probable sequence of build up of deposit could be seen. Here, the edge of the colony became broken up into a series of satellite centres of oxidation, the ultimate satellites being single cells encrusted by manganese oxides. Outwards from these single, encrusted cells were new buds which were quite free of deposit (Fig. 27), the whole pattern suggesting that the formation of a central mass of manganese oxide in the



a b

c

Fig. 26. Colonies of Hyphomicrobium sp. isolated from Derwent pipelines on PC medium, showing dark centres of oxidized manganese and clear halos of unencrusted cells.

a) Slight oxidation and wide halo.

b) Denser colony with more extensive oxidation.

c) Edge of colony showing stalked cells projecting from the edge, with unencrusted buds.

Fig. 27. Edge of a colony of Hyphomicrobium oxidizing manganese on medium PC, showing encrusted cells forming satellite centres of oxidation, and other, presumably newer buds which are as yet unencrusted.

colony resulted from the confluence of satellite centres at the advancing edge. The unencrusted cells would presumably become encrusted at a later stage of development, while new buds would be formed on the outside.

The morphology of the Hyphomicrobium cells in manganese-oxidizing colonies varied considerably. In some colonies the cells had the classical pear-shape (Bergey, 1957) of Hyphomicrobium vulgare Stutzer et Hartleb, with long unbranched stalks (Fig. 25c). In other colonies the stalks exhibited varying degrees of branching, while the cells became swollen with refractile granules of poly β-hydroxy-butyrate. In the most bizarre form the cells were grossly distorted and the stalks repeatedly branched to form a network ramifying through the manganese oxide (Fig. 28). Very similar networks were found in deposits from the Derwent pipelines.

Platings of pipeline deposit were not always successful. It appeared to depend on the condition of the deposit sample, for if sampling was carried out carefully, by scraping only the very surface of the fresh, wet deposit into a sterile dish and refrigerating until plated, success could generally be assured. When plated out in serial dilution more than 10^5 colonies of manganese-oxidizing hyphomicrobia were obtained per wet gram of deposit. However, the actual numbers of hyphomicrobia could be much higher than this

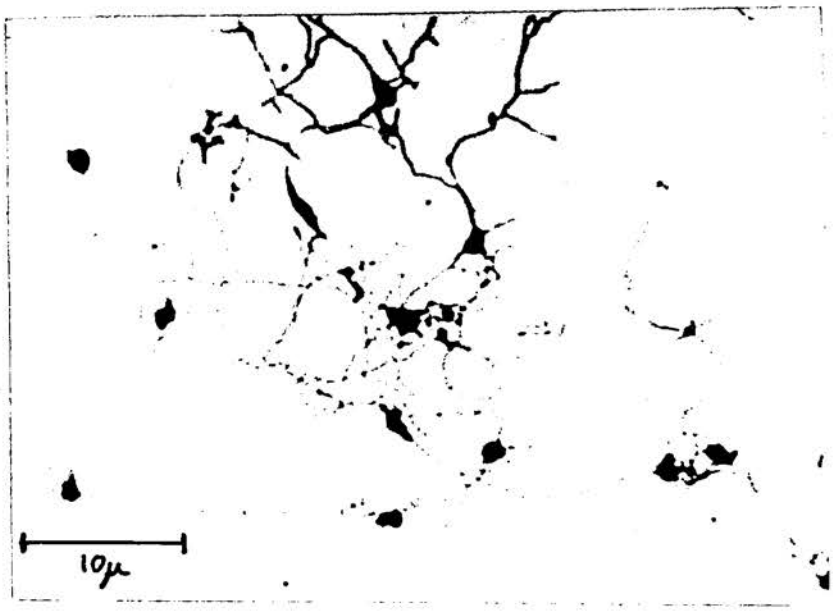


Fig. 28. Bizarre network form of Hyphomicrobium isolated from Derwent pipelines on PC medium. Manganese oxides have been dissolved away with 5% oxalic acid, the agar leached away with hot water and the cells stained with carbol fuchsin.

since it was impossible to grind the deposit finely enough to release all cells as separate units. From the fact that manganese-oxidizing hyphomicrobia could be isolated in high numbers from pipeline deposits and the fact that such bacteria ramify as a network throughout the actual deposits, it was concluded that this pleomorphic Hyphomicrobium is overwhelmingly responsible for the oxidation and deposition of manganese in the Derwent pipelines.

Colonies of hyphomicrobia from serial platings of deposits were ground and replated on media PC and 337MH. On replating, growth was sporadic and, after two or more transfers, the power to oxidize manganese was lost. One strain, Hyphomicrobium T37, was isolated in pure culture.

The deposits which formed when various lake waters were circulated in the laboratory apparatus were also ground and plated. As in the case of pipeline deposits, the types of microorganisms which oxidized manganese on PC medium included fungi, rod-shaped bacteria and hyphomicrobia. In addition an actinomycete was isolated. Hyphomicrobia were consistently isolated in large numbers from these deposits, providing additional proof that they are the principal manganese-oxidizing organisms. Circulation of water from various localities in Tasmania showed that the hyphomicrobia are widely distributed. This was true even in waters to which soluble manganese had to be added

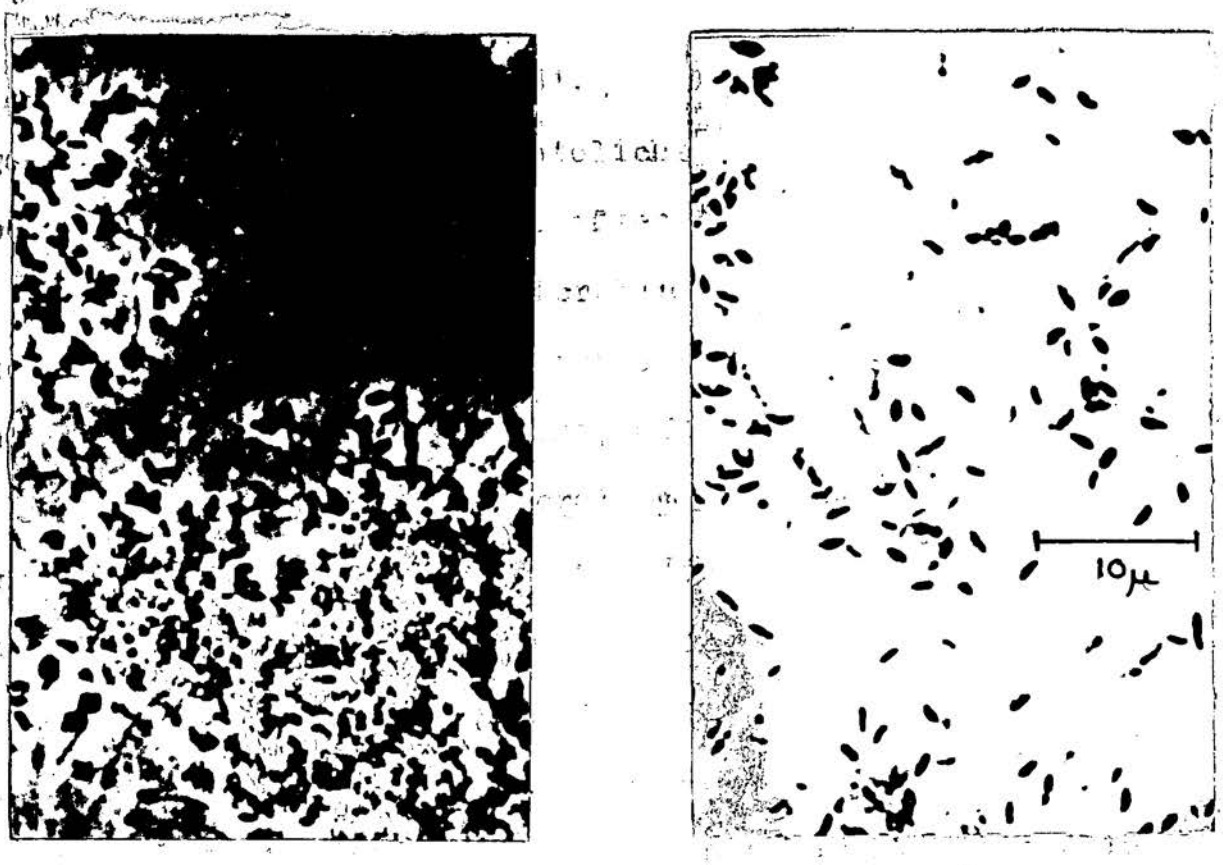
to produce a deposit in the apparatus.

6. Morphology and taxonomy of Hyphomicrobium T37

(a) Pleomorphy

Colonies of manganese-oxidizing hyphomicrobia isolated from pipeline deposits were ground up and replated on various media. They did not always grow after transfer and lost the ability to oxidize manganese after two to four replatings. The isolate designated T37 was obtained in pure culture and was used for studies on morphology. An astonishing degree of pleomorphy was noted in cells from this culture grown on various media and observation of hyphomicrobia in natural habitats revealed that the same degree of pleomorphy occurs in natural conditions.

The normal Hyphomicrobium is a pear-shaped or bean-shaped cell, about 1.0-2.0 μ long x 0.5-0.7 μ wide, with a slender, unbranched stalk about 0.2μ-0.3μ in diameter, at the end of which a motile bud develops (Hirsch and Conti, 1964; Zavarzin, 1961b). Cells with this classical morphology were characteristic of medium 337 (Fig. 29), but often they were observed on other media. Similar cells were observed in a variety of natural habitats, including on freshwater plankton and in mucilages and jellies (Fig. 30) produced by colonial diatoms, blue-green



a b

Fig. 29. Classical morphology of Hyphomicrobium T37, showing regular shape of cell and unbranched stalks.
a) Undisturbed colony on surface of 337 Medium.
b) Stained cells from a colony grown on 337MH.

Fig. 30. Classical morphology of Hyphomicrobium in an algal jelly showing regular, pear-shaped cells with sparingly branched stalks.

algae, Chlorococcales and protolichens. In the pipeline deposits classical cells were often present.

Pleomorphy in the hyphomicrobia observed in this study takes two forms - firstly, a range of branching of the stalks and, secondly, a bizarre, often contorted cell shape.

In pure culture, Hyphomicrobium T37 exhibited varying degrees of pleomorphy. On PC and 337MH media, cells usually were pleomorphic (Figs. 31-33) with a tendency to become giant, lobed and swollen with refractile granules of poly-β-hydroxybutyrate (Hirsch and Conti, 1964). Extremely bizarre forms are difficult to recognize as hyphomicrobia (Figs. 32 and 33). Bizarre cells often bore regular pear-shaped buds at the ends of stalks (Fig. 33). Sometimes a motile swarmer, which has remained attached, may be seen towing its bizarre parent. A range of bizarre cell shape may be accompanied by varying degrees of branching of the stalks. Figs. 28 and 34 show portions of colonies where pleomorphy is evident both in the bizarre cell shape and in the extreme reticulation of the stalks. This form resembles closely Aristovskaya's illustrations of Pedomicrobium (Aristovskaya, 1961). However, as Hyphomicrobium T37 in pure culture displays morphological variation ranging from the classical form of Hyphomicrobium vulgare to the bizarre network described as Pedomicrobium, it is clear that the latter is but a morphological form of Hyphomicrobium and

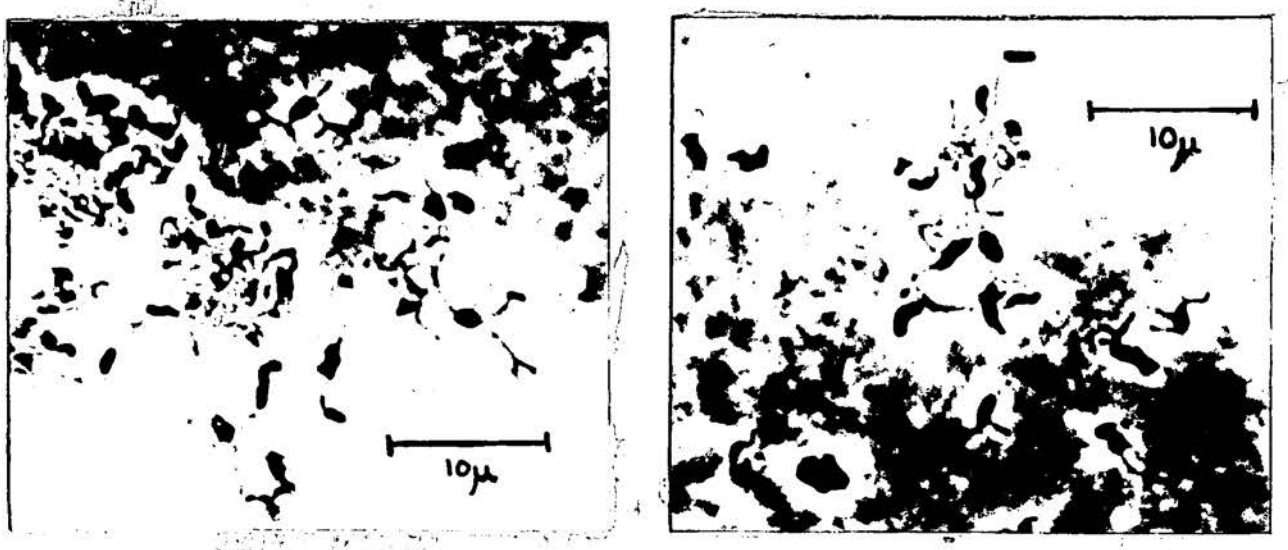


Fig. 31. Hyphomicrobium T37, showing varying degrees of bizarre cell shape, and branching of stalks. Cells from disrupted colony in 337MH medium. Phase contrast.

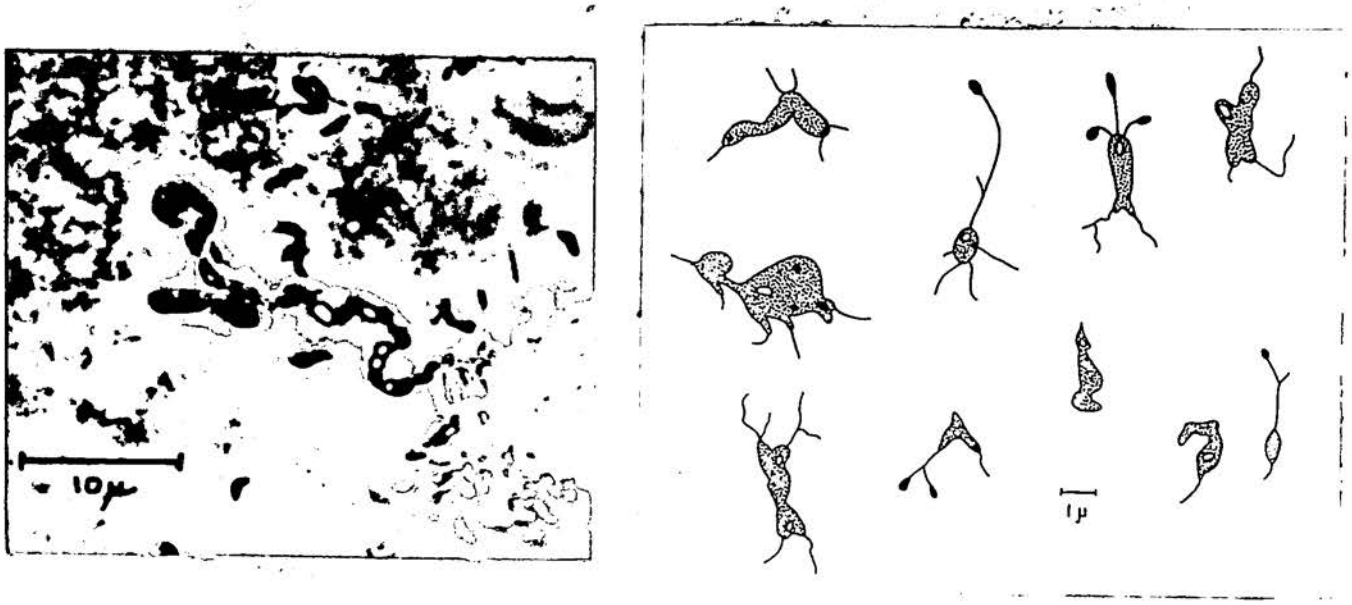


Fig. 32 (left). Hyphomicrobium T37, showing extremely bizarre cell form. The sausage-shaped cell is swollen at intervals with granules of poly β-hydroxybutyrate. Live cells at edge of colony in 337MH medium.

Fig. 33. (right) Hyphomicrobium T37 showing bizarre cells, often bearing classical, pear-shaped buds. Camera Lucida drawings of live cells in 337MH medium.

84.

that the genus Pedomicrobium is invalid. Observations by phase contrast microscopy showed that pedomicrobia may form between some strains of the genera.

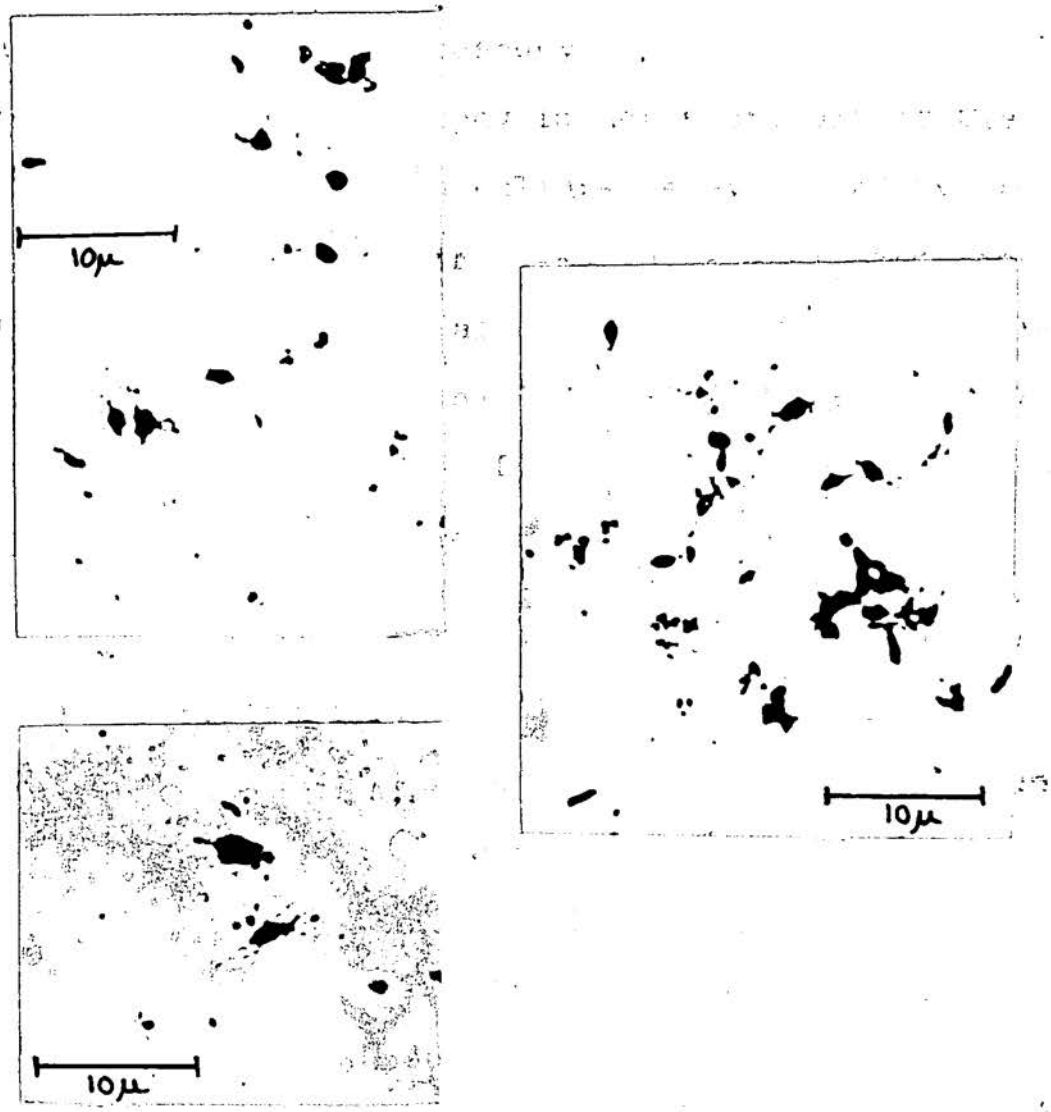


Fig. 34. Hyphomicrobium T37, showing a range of cell shape and considerable branching of stalks. Smear of cells from a disrupted, non-oxidizing colony on PC medium. Stained with carbol fuchsin.

that the genus Pedomicrobium is invalid. Observations by phase contrast microscopy suggest that anastomoses may form between some stalks of the network. However, this requires confirmation by electron microscopy.

That this bizarre pleomorphy is not a product of the artificial conditions of pure culture is evidenced by the observation of closely similar forms in natural situations. In a jelly produced by colonial diatoms, pleomorphy in the form of both multiple branching and bizarre cell shape was regularly observed (Fig. 35). In addition, forms with the "Pedomicrobium morphology" are found regularly in natural pipeline deposits (Fig. 36).

(b) Ultrastructure

In view of its pleomorphy, Hyphomicrobium T37 was examined by electron microscopy. Thin sections, after osmium fixation and lead post-staining, confirmed the ultrastructural details reported by Conti and Hirsch (1965) for their strains of Hyphomicrobium. Figures 37 and 38 show the well-known structural features such as poly β-hydroxybutyrate reserves in cells, the continuity of cytoplasm, cell membrane and cell wall in cell and stalk, and the presence of DNA in mother cell and bud. The prescence of DNA in the stalk (Fig. 39) suggests that DNA migrates from mother cell to bud during reproduction by budding. The cell wall is the double membrane

Fig. 35. Pleomorphy in natural environments - Hyphomicrobium in an algal jelly showing multiple branching of stalks (camera lucida drawing through several planes of focus).

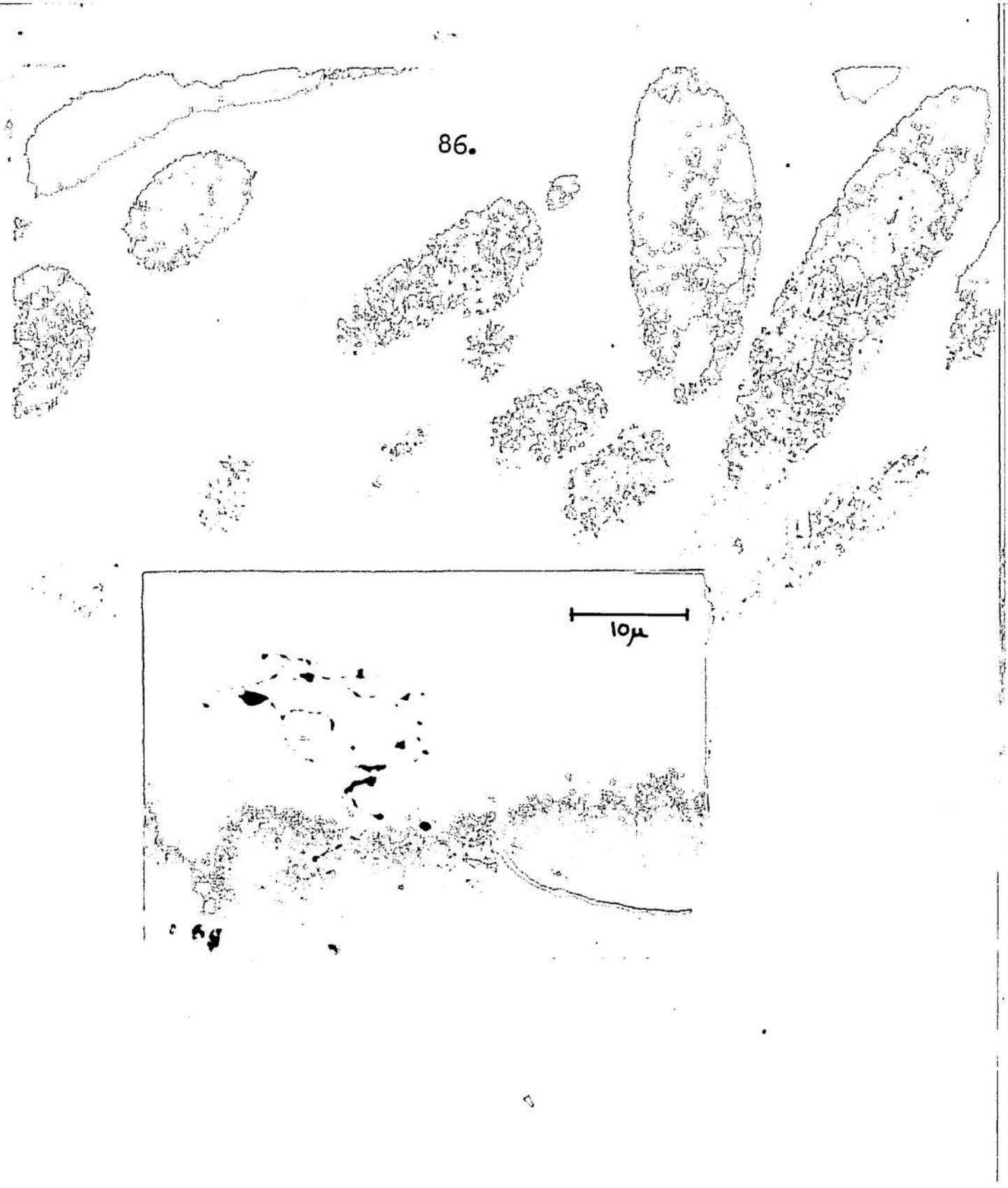


Fig. 36. Hyphomicrobium from Derwent pipeline deposits showing "Pedomicrobium morphology". Carbol Fuchsin stained, after removal of manganese oxides with oxalic acid.

Fig. 37. Hyphomicrobium T37 in thin section, showing continuity of cell wall and cytoplasm in cell and stalk, and the presence of DNA in mother cell and bud. One stalk has been cut in cross section (arrowed). Other cells show accumulation of poly B-hydroxybutyrate (double arrows). X c.37,000. Courtesy of Dr.Y.T. Tchan.

Fig. 38. Hyphomicrobium T37 in thin section, showing ultrastructural features similar to those in Figure 37.

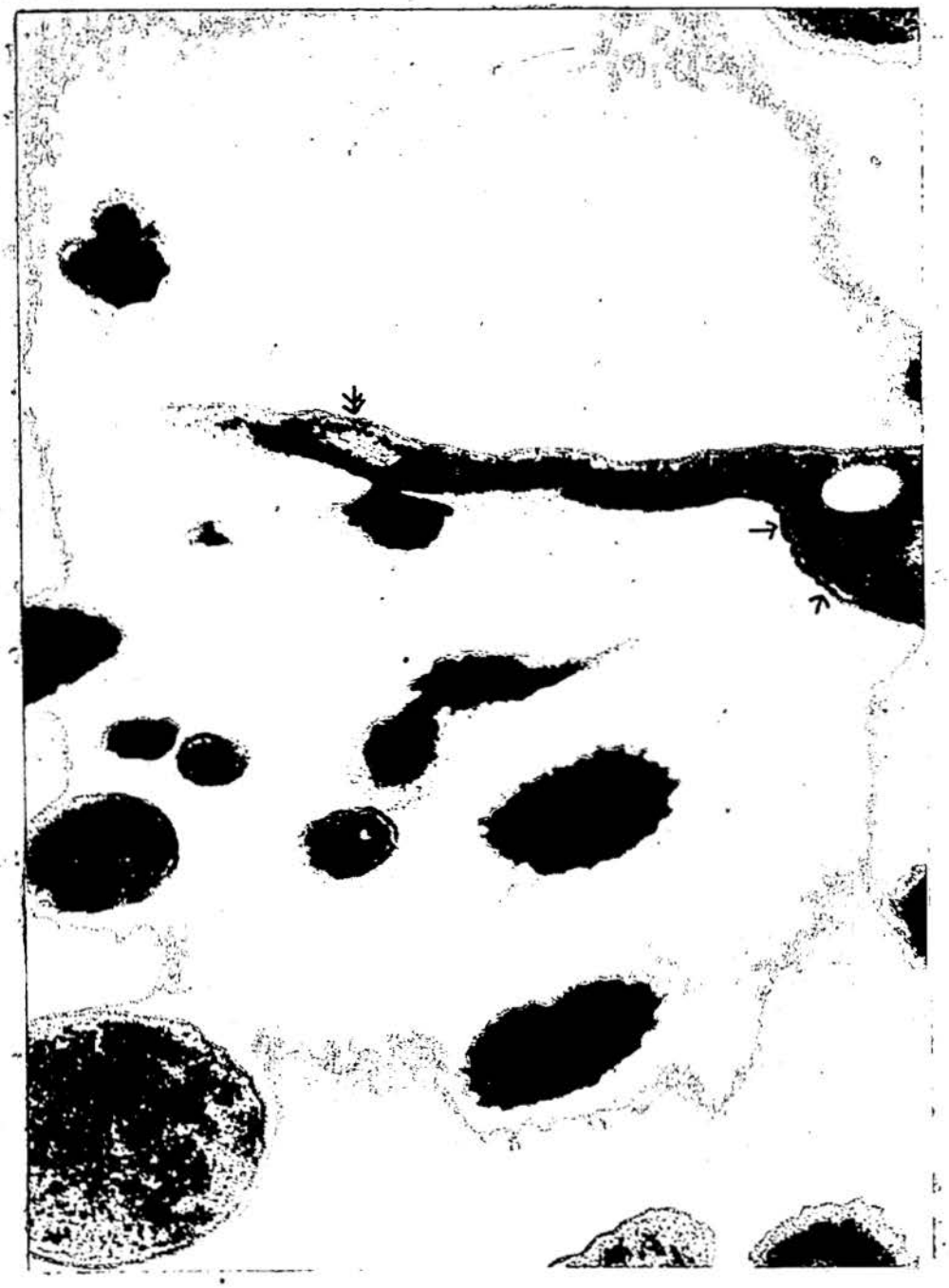


Fig. 39. Thin section of Hyphomicrobium T37, showing the double-membrane type of cell wall (arrows) and the presence of DNA in the stalk (double arrow).
Micrograph by courtesy of Dr. Y.T. Tchan.

type characteristic of gram-negative bacteria (Salton, 1964), the cytoplasm being bounded by two multilayered, parallel membranes (Fig. 39). This agrees with the findings of Conti and Hirsch (1965) for Hyphomicrobium and Rhodomicrobium. However, the intracytoplasmic membranes reported by those authors for most of their strains are not apparent in the micrographs of T37 though they could be masked by the densely-packed ribosomes.

The pleomorphic nature of T37, detected by light microscopy, is confirmed by electron microscopy. The production of several stalks from one cell is shown in Fig. 40 while in Figs. 41 and 42 stages in the development of the colonial form can be seen. Figure 42 also shows the tendency for bizarre shape, one cell having a Y-shape which was also observed by light microscopy. One interesting feature of T37 is the occurrence of apparently large numbers of flagella-like, or fimbrae-like appendages (Figs. 41 and 42). These appear to be borne not only on the cells but also on the stalks (Figs. 43 and 44). The cell shown in Fig. 45, which appears to have six flagella-like appendages, could possibly have been a motile swarmer. The possibility that these appendages play some part in the mechanism of adherence to the pipeline walls is considered in the Discussion.

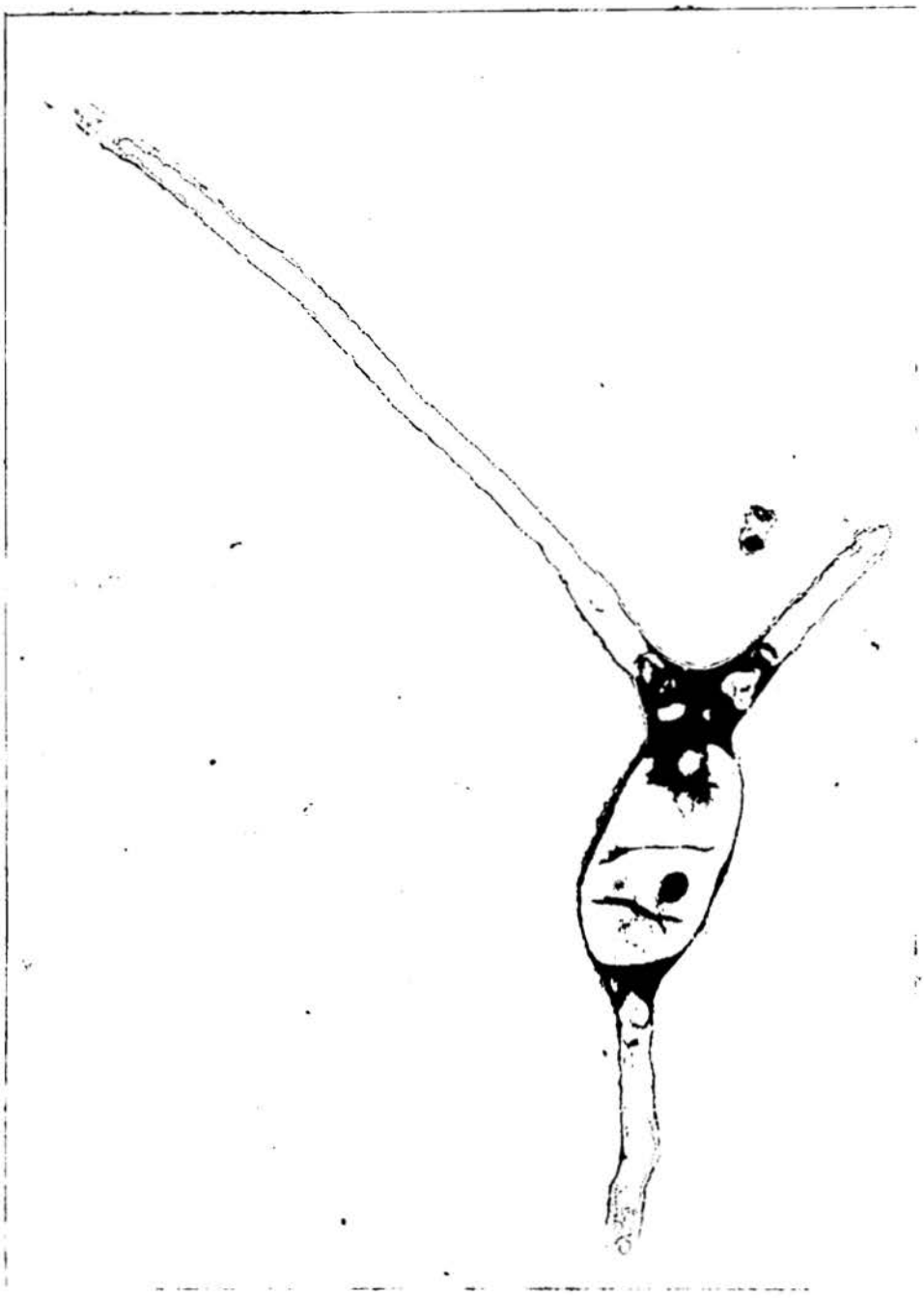


Fig. 40. Negatively-stained preparation of Hyphomicrobium T37 showing production of several stalks from one cell.
Micrograph by courtesy of Dr. Y.T. Tchan.

Fig. 41. Negatively stained preparation of Hyphomicrobium T37 showing numerous flagella-like appendages and stages in development of the colonial form by multiple budding (arrowed).

Fig. 42. Negatively stained preparation of Hyphomicrobium T37, showing formation of several stalks per cell, numerous flagella-like appendages, and a Y-shaped cell (arrowed).

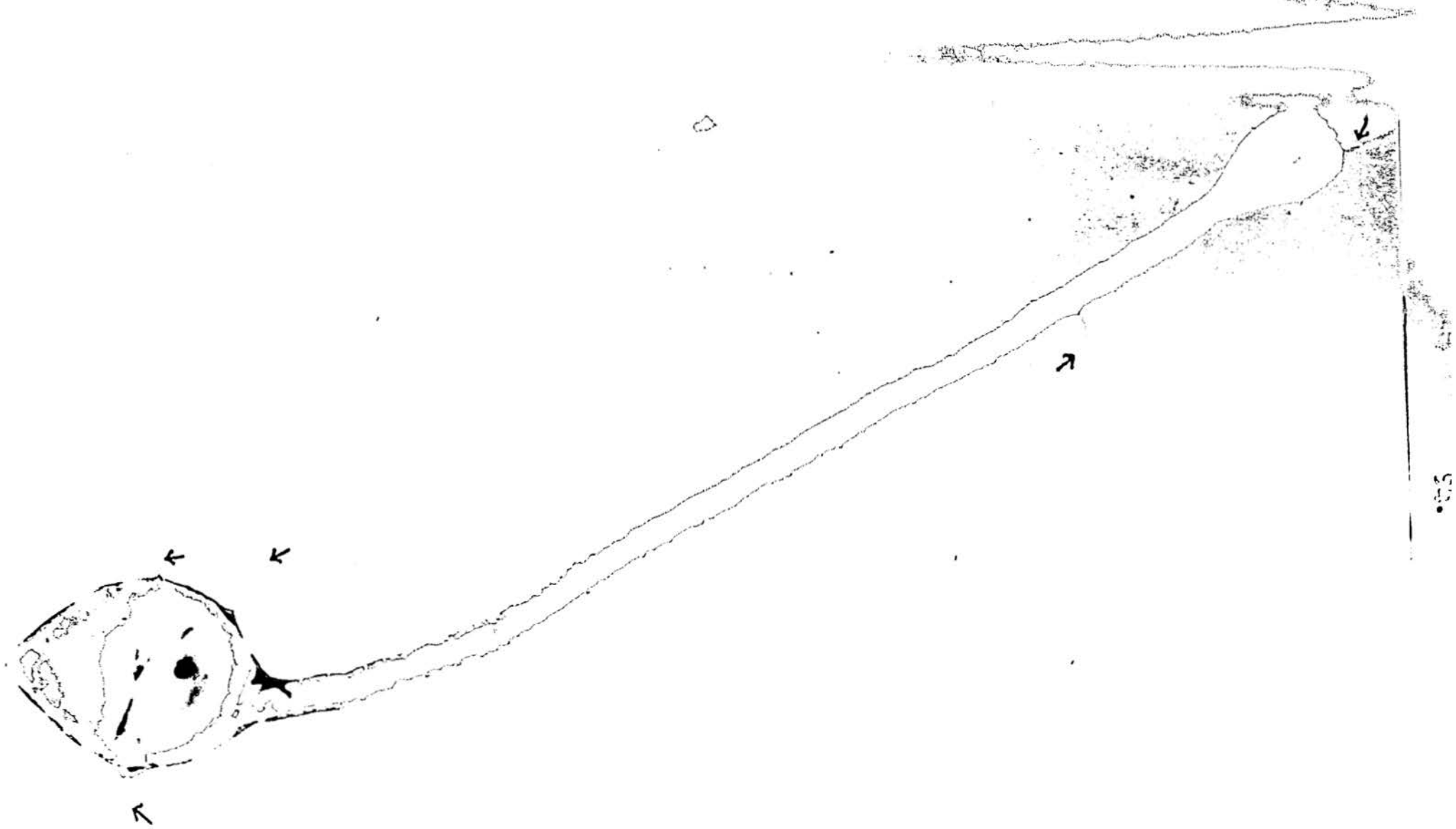


Fig. 1. Negatively stained preparation of Hyphomicrobium T37, showing flagellum-like appendage (arrowed) borne on stalk and cell. Micrograph by courtesy of Dr. J.F. Pate...

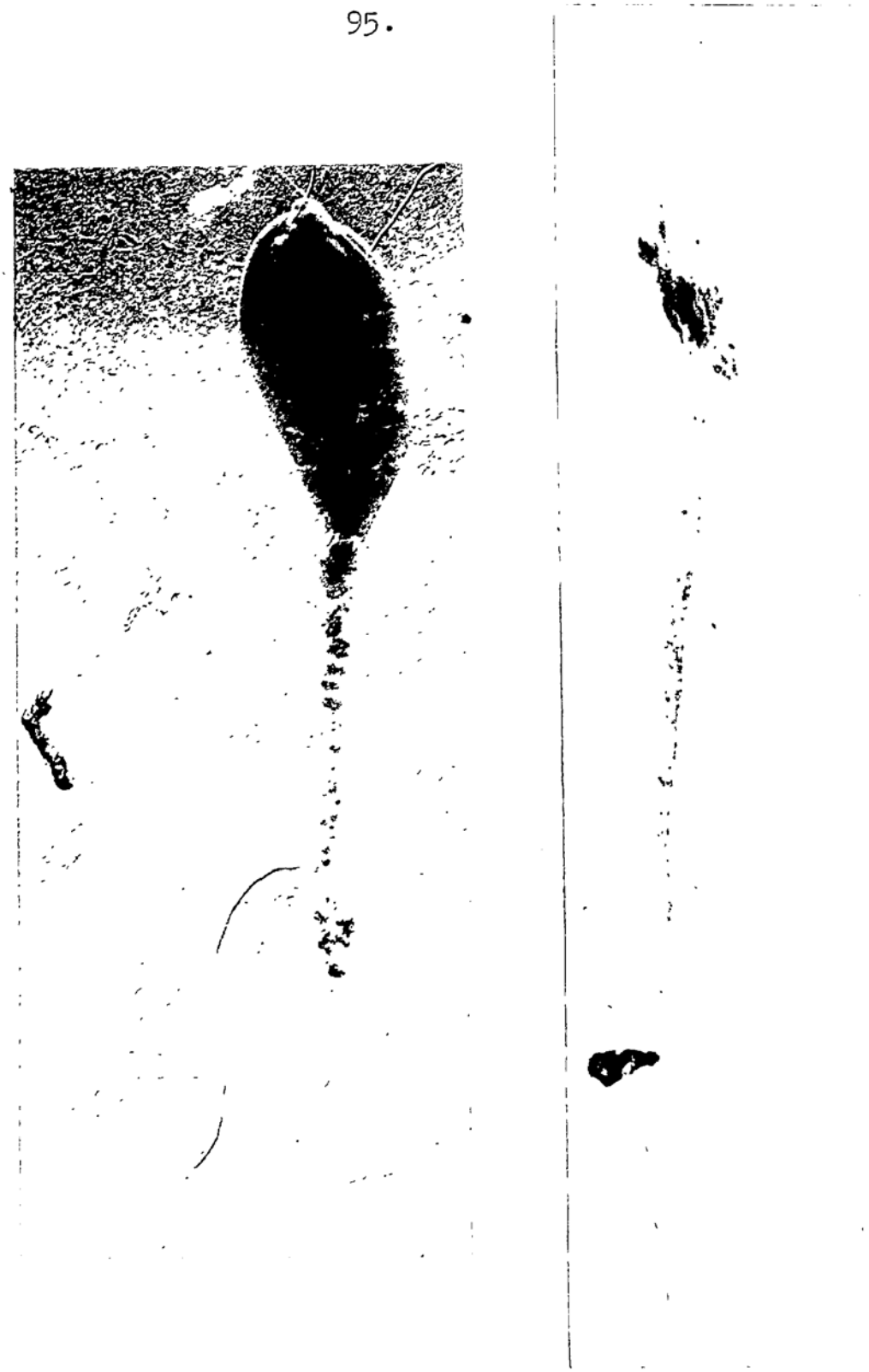


Fig. 44. Gold-palladium shadowed preparation of Hyphomicrobium T37, showing occurrence of flagella-like appendages on stalk and cell.

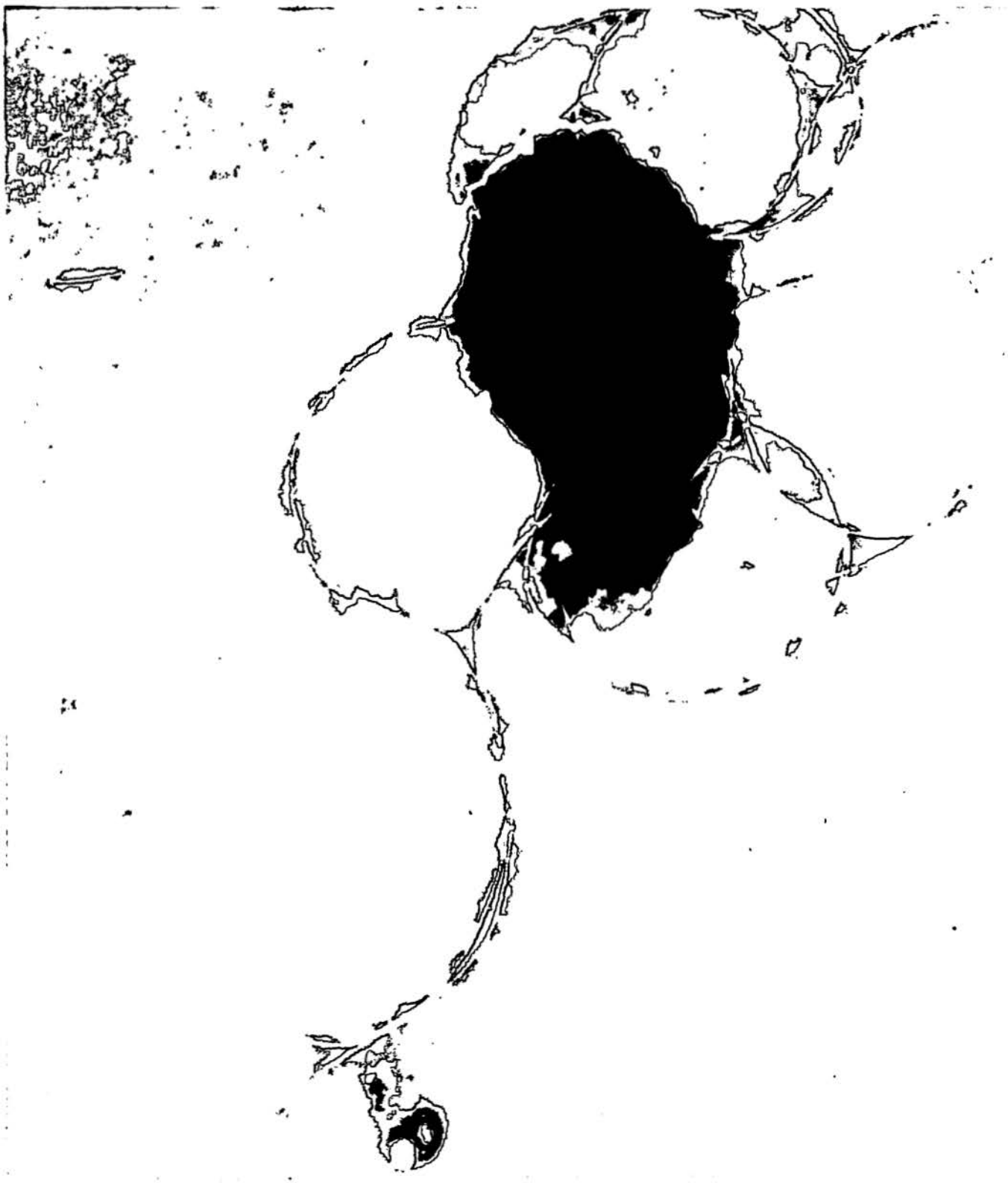


Fig. 45. Negatively-stained preparation of Hyphomicrobium T37. The cell is probably a motile swarmer and appears to bear six flagella-like appendages.

7. Mechanism of attachment to surfaces

When considering the mode of attachment of bacteria to the pipeline surface, the possibility of electrostatic attraction was investigated. The pipeline is likely to have a negatively charged surface so that any microorganism having a positively-charged surface would seem to possess a selective advantage for electrostatic adsorption at the pipe-surface. The pattern of electrophoretic mobility (Fig. 46) of Hyphomicrobium T37 shows that the mobility rises rapidly from zero at pH 2.0 to a steady value at pH values above 4.0. The shape of the curve resembles that for certain rhizobia (Marshall, 1967) and Aerobacter aerogenes (Plummer and James, 1961) and indicates that the net surface charge is negative and due entirely to the dissociation of surface carboxyl groups. In view of the negative surface charge it seems unlikely that Hyphomicrobium T37 attaches to the surface by direct electrostatic attraction.

Of considerable importance in any study of the interaction between the hyphomicrobia and any solid surface is the actual point of attachment to the surface. In Caulobacter the point of attachment is the stalk and this genus forms rosettes by apposition of these stalks (Poindexter, 1964). In Hyphomicrobium, on the other hand,

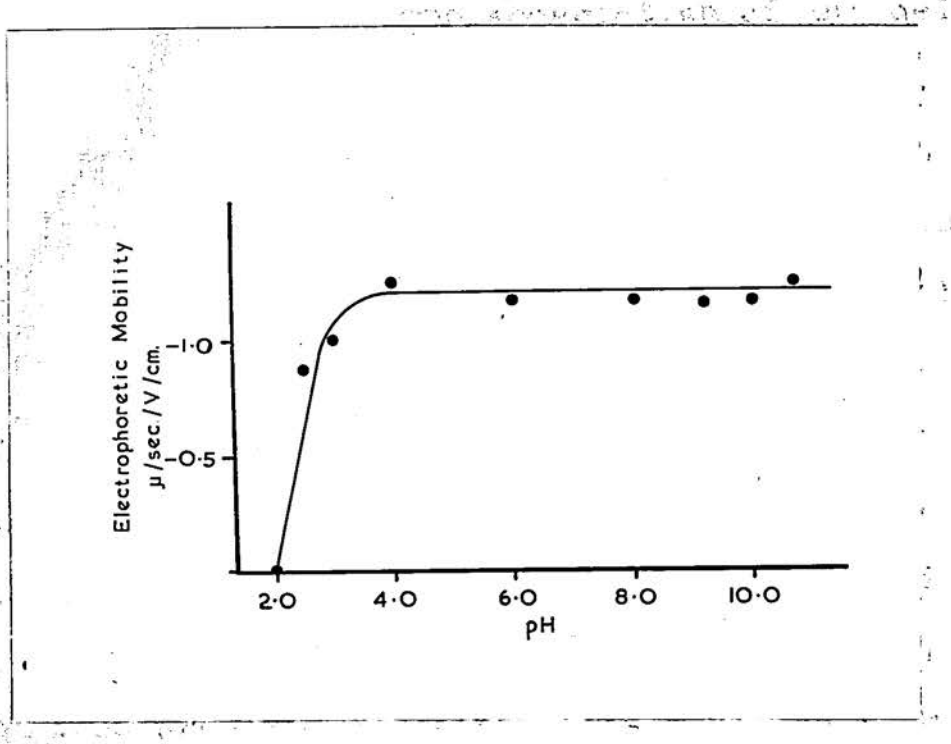


Fig. 46. pH-electrophoretic mobility curve for Hyphomicrobium T37.

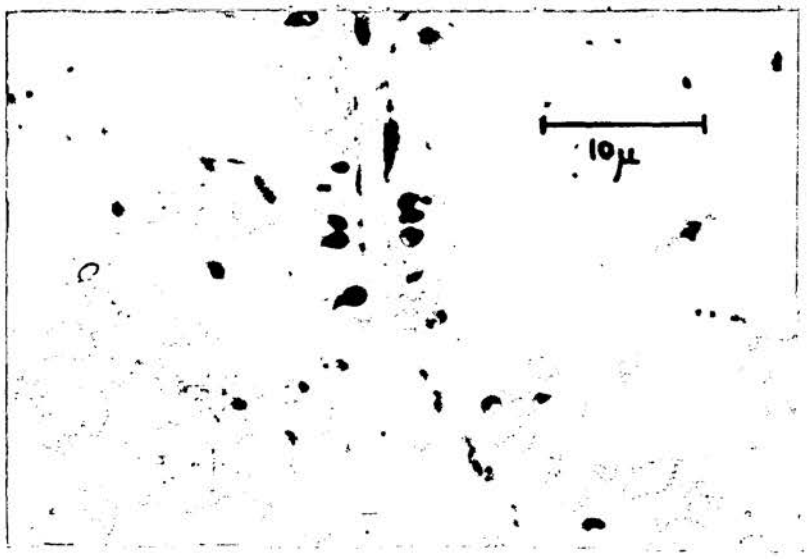


Fig. 47. Hyphomicrobium cells aligned at right angles to the fungal hypha to which they appear to be adhering.

rosette formation involves association of the cell portion, with stalks spreading radially (Zavarzin, 1961b). In a natural situation, hyphomicrobia associated with fungal hyphae were observed. The cells were all orientated at right angles to the hyphae, with the cells possibly being adsorbed to some transparent, extracellular component of the fungal hyphae (Fig. 47). This suggests that hold-fast material may be secreted by the broad end of the Hyphomicrobium cell, as in the case of rosette formation (Conti and Hirsch, 1965). Attempts to reproduce this form of adsorption under controlled conditions, using glass wool and a pure culture of Hyphomicrobium T37, were not successful.

G. DISCUSSION

The marked difference in the degree of manganese deposition in the Derwent pipelines compared with those of Great Lake at once suggested that there was a difference in manganese-availability in the two lakes or their catchments. The details of the Tasmanian case fitted in with the popular theory that manganese problems are associated with brown, humic waters rather than clear ones. For these reasons it was thought that a comparative study of the limnology of the two lakes may reveal the reasons for the difference and thus give some understanding of the nature

of the problem in Lake King William where it is acute.

The fact that supplementing Great Lake water with sterile manganous sulphate produces a deposit in the recirculatory apparatus shows quite clearly that the difference between the two lakes is one of manganese availability and not lack of appropriate manganese bacteria in Great Lake. Despite the unsatisfactory nature of the manganese analyses they too show that Lake King William does contain more manganese than does Great Lake. However, in both lakes the manganese levels are very low.

On the basis of inorganic ions Lake King William and Great Lake appear to be little different qualitatively or quantitatively. Both lakes are very soft, with a low total concentration of nutrients, low alkalinity and a slightly acid pH. Further, they do not appear to be markedly different in the composition of their plankton populations, and from these aspects there is nothing to suggest a reason for the difference in manganese availability.

Both lakes are somewhat unusual in that they do not develop a lasting stratification. This is clearly shown by their thermal properties and the oxygen-saturation of the waters. The reason for this lack of stratification is the exposed situation of these lakes on an elevated plateau with high wind frequency. Though detailed lake temperature readings are available for one year only, meteorological

data show that stratification would be very unlikely in any year. This suggests that solution of manganese by anaerobic reduction in the hypolimnion, which is common elsewhere in the world, is unlikely to take place in these two lakes.

Great Lake lies in a depression in the Jurassic dolerite of the Central Plateau of Tasmania. Along the south-western shore and in isolated areas elsewhere along the shoreline, Tertiary basalt is exposed (Banks, 1965). Dolerite contains an average of 0.15% manganese (McDougal, 1962) and Tiller (1964) has shown that much of the manganese derived from weathering of dolerite passes into solution under waterlogged conditions. Great Lake is surrounded by large tracts of open sclerophyll forest dominated by Eucalyptus spp. and by high moorland heath dominated by Epacridaceae and Restionaceae (Jackson, 1965). The Jurassic dolerite extends westwards across the central plateau to the eastern edges of Lakes St. Clair and King William and here essentially the same vegetation occurs as in the Great Lake area. The western shores of both Lake King William and Lake St. Clair, however, are bounded by Quaternary deposits and here the vegetation changes to temperate rain forest, dominated by Nothofagus cunninghamii and Atherosperma moschata. On flatter land the characteristic sedgeland of button grass (Gymnoschoenus sphaerocephalus)

develops. Thus, as there is abundant dolerite around both lakes, it seems likely that there is geologically as much manganese in the Great Lake catchment as in that of Lake King William. However, the availability of this manganese is almost certainly affected by the differences in soils and vegetation of the two catchments, associated with some differences in geology and marked differences in rainfall patterns (Langford, 1965).

In this context the higher organic content of Lake King William is of interest. The higher organic status is shown by the higher levels of organic carbon and colour, and the lower transparency. The origin of this organic material is the humic substances which flow into the lake from the surrounding button grass plains. The largest areas of button grass surround the Guelph Arm and the western edge of Lake King William and it is noticeable that the Guelph Arm consistently has the highest colour rating. The button grass around Lake St. Clair is restricted to the small river valleys on the Western side and contributes relatively little coloured water to the large volume of the lake. Accordingly, the water is usually clear and colourless. The fluctuating values for colour in the main arm of Lake King William are probably due to the relative volumes of clear water coming from Lake St. Clair and coloured water from the button grass plains to the west of Lake King William.

The humic materials which flow into Lake King William from the surrounding catchment do offer a possible source for the manganese; the association of manganese problems with brown, humic waters is a well-known phenomenon in many parts of the world. Because the principal trouble area - Lake King William - is the only lake with humic waters and with extensive, peaty Gymnoschoenus plains in its catchment, and in the absence of an explanation based on stratified lakes, it might be assumed that the root of the problem in Lake King William lies in the solution of manganese by complexing or chelation with organic leachates in these plains. However, the fact that supplements of manganous sulphate to Great Lake water cause deposition of manganese suggests an alternative explanation based on solution of uncomplexed, divalent manganese brought about by waterlogging in the soils of the plains. The whole problem of manganese availability is in need of further study.

Many investigators have linked manganese deposition in pipelines with the activity of microorganisms. However, their arguments have always been based, reasonably, on observation of appropriate bacteria within the deposits and their isolation in synthetic media. In this study, the inhibition of oxidation by azide treatment and autoclaving of the water is experimental proof that micro-organisms are involved, at least in the early stages.

In the case of autoclaved water, the fact that inoculation with untreated water produces a deposit possibly could be explained on the basis of addition of existing manganese oxides which could act as a surface for adsorption and chemical oxidation of manganous ions. However, in the presence of azide, inoculation with untreated water fails to produce a deposit, indicating that the inhibitory effect of both azide and autoclaving is that of killing or preventing growth of the manganese-oxidizing microorganisms. These results agree well with similar experiments in soils (Mann and Quastel, 1946).

The effect of temperature on the rate of build-up of deposit in the laboratory apparatus is that which would be expected for biological or chemical reactions. However, the periodic variation in tests of Great Lake water and King William water are sporadic and do not fit in with the seasonal temperature changes. They do appear to be correlated with variations in manganese concentration in these lakes, but the whole field of manganese cycles in the lakes and catchments is in need of critical study.

The use of the recirculatory apparatus to predict the possibility of manganese deposits developing in pipelines when new power schemes are installed is limited by the above considerations. Absence of deposit in any single test would not necessarily mean that the particular body

of water was unlikely to be troublesome. Tests would have to be performed regularly to allow for seasonal or periodic variation in manganese availability. Further, a series of negative tests with river water would give no guarantee that a manganese problem would not develop if the river was impounded. This is particularly true since, frequently, the ideal dam site lies in a deep narrow gorge. Impoundment of the river at points such as this is likely to produce a deep lake protected from wind action by the walls of the gorge, so that the anaerobic conditions favouring solution of manganese are very likely to occur. On the other hand, tests with waters from button grass areas suggest that flooding or waterlogging of Gymnoschoenus plains would probably create a manganese problem.

A review of the literature suggested that the organisms producing manganese deposits in Tasmanian pipelines would be chlamydobacteria and a determined search for these bacteria during the early part of this study delayed recognition of the true cause. However, it became apparent that chlamydobacteria were so rare in the deposits that they could not possibly be the major cause. Similarly, fungal hyphae are present in the deposit but not in sufficient numbers to be of significance. There can be no doubt, however, of the overwhelming importance of Hyphomicrobium in Tasmanian deposits. The facts that the deposits are

completely ramified by the network of stalks and cells, that manganese-oxidizing hyphomicrobia are isolated in high numbers on artifical media, and that the same organisms produce a deposit in the laboratory apparatus, are conclusive evidence for the involvement of this bacterium.

Analyses show that deposits from various parts of the world are quite similar in their composition. This suggests that they could be deposited by the same species of micro-organisms or by the same process. Because hyphomicrobia are difficult to observe and are not well-known to most microbiologists, it has been suggested (Tyler and Marshall, 1967b) that Hyphomicrobium may be more widespread in manganese deposits than has been realised and that it could be the true cause even in cases where chlamydobacteria have been blamed. This view was strengthened by the fact that manganese deposits from pipelines at Kareeya, Queensland, are also dominated by Hyphomicrobium and that a very similar soil organism, the so-called Pedomicrobium, oxidizes manganese (Aristovskaya, 1961). The abundance of chlamydobacteria in the Tumut deposits, which have high manganese and low iron contents, appears to be an argument against this idea. However, for this to be so the role of chlamydobacteria as manganese-oxidizers in Tumut deposits would have to be proved by the same rigorous criteria which showed the importance of hyphomicrobia in Tasmanian deposits.

It will be instructive to compare critically the deposits from various parts of the world to determine the relative importance of hyphomicrobia and chlamydobacteria.

The extent to which rod-shaped bacteria contribute to the formation of pipeline deposits is uncertain. Microscopical examination is of little value in the absence of distinctive morphology. However, despite the fact that the organism designated T48 oxidizes manganese vigorously in pure culture, the low frequency of this type of bacterium in plate counts suggests that it is not of major consequence.

The exact nature of Metallogenium symbioticum remains a problem. The same tapering threads described by Zavarzin (1961a) are regularly found in association with manganese-oxidizing fungi isolated in Tasmania. This is true even when the fungus is grown in liquid culture, suggesting that the threads are not artifacts produced by diffusion of metabolites from the fungal hyphae. However, the nature of the "organism" as described by Zavarzin is puzzling; even its dimensions (about 200A° diameter - Zavarzin, 1963) pose problems in cellular organisation. As fungi are considered to be unimportant in Tasmanian deposits, the question of Metallogenium is not considered further.

Hyphomicrobium is usually envisaged (Bergey's Manual, 1957) as a pear-shaped cell reproducing by the production of a bud at the end of a long, unbranched stalk. Variation

from this classical morphology has recently been recorded and the present investigation confirmed that this tendency towards pleomorphy is widespread both in natural environments and in pure culture. The illustrations presented here show morphological forms ranging from the classical hyphomicrobial shape, through types with regular cells but reticulate stalks, to cells showing bizarre shapes with or without a reticulate stalk system. The stalk has always been regarded as an essential part of the reproductive process and its role in the production of motile buds has been amply demonstrated. In this context, branching of the stalks should increase the capacity for bud formation and enhance formation of the colonial organization.

In many cases, bizarre cell shape appears to result from the production of several stalks at different points on the cell. A narrowing bulge occurs in the direction in which each stalk arises. However, the occurrence of multiple branching does not necessarily imply bizarre cell shape. When a stalk branches shortly after leaving the cell, or when several stalks arise at the same point, cell shape is commonly classical.

In many cells giantism and bizarre shape appears to be related to accumulation of large poly-β-hydroxybutyrate reserves, a phenomenon also reported by Hirsch and Conti (1964). It is interesting to speculate on the nature of

the cell wall which allows this variation in cell shape. In this context, it is noteworthy that Vincent and Colburn (1961) found that calcium deficiency in Rhizobium trifolii led to enlarged and distorted cell shapes. However, there is no possibility of calcium deficiency in 337 media and the apparent plasticity of the cell wall must be explained on other grounds.

During the present investigation a whole range of morphological types, from the classical Hyphomicrobium to Aristovskaya's Pedomicrobium (Aristovskaya, 1961) was observed in budding bacteria both in pure culture and in natural environments. Aristovskaya noted a morphological relationship between her Pedomicrobium and the anaerobic, photosynthetic Rhodomicrobium but she did not comment on possible relationships with Hyphomicrobium. Because of the fact that Hyphomicrobium T37 in pure culture exhibited the complete range of variation mentioned above, it has been suggested that the genus Pedomicrobium is invalid and that it should be regarded as a form of Hyphomicrobium (Tyler and Marshall, 1967c). The close morphological similarity between Hyphomicrobium T37 and "Pedomicrobium" is complemented by their ability to oxidize manganese. It seems likely that the same bacterium is involved in both cases. In view of the wide variety of morphological forms observed in their cultures, Hirsch and Conti (1964) suggested a

complete re-evaluation of the budding bacteria. The variation reported in this investigation sounds a further note of warning; a cautious approach to erecting new genera of budding bacteria may save future confusion.

The ultrastructure of hyphomicrobia has not been extensively studied and the details for T37 presented here are useful in confirming previous work (Conti and Hirsch, 1965). The apparently numerous flagella- or fimbrae-like appendages are a source for speculation. The appendages do not have the sinusoidal appearance frequently exhibited by bacterial flagella (Houwink and van Iterson, 1950; Hoeniger, 1965). However, flagella do not always display this feature clearly (e.g. Poindexter, 1964, Fig. 8; Houwink and van Iterson, 1950, Fig. 6) and as Hyphomicrobium is known to produce flagellate swarmers the flagellar nature of these appendages is highly likely. Their appearance in shadowed preparations is more like that of flagella than of fimbrae (= pili - Duguid and Anderson, 1967). However, fimbrae are well known for their powers of adsorption. Brinton (1965) points out that fimbriate cells of E. coli will adhere to almost any surface or to other cells, that they form pellicles at air-water interfaces and that they agglutinate red blood cells. All these properties are ascribed to the hydrophobicity of the fimbrae. Fimbrae are also the sites for attachment

of male-specific phages in E. coli (Brinton, 1965) and Ishibashi (1967) has shown that the "F pilus" is probably the structural entity of f^+ antigenicity of E. coli. In view of their possible significance as organs of attachment, the exact nature of the filamentous appendages of T37 should be investigated.

Other possible mechanisms for attachment of manganese-oxidizing bacteria to pipe surfaces have been considered. Direct electrostatic attraction is unlikely in the case of T37 since its surface is negatively-charged, suggesting repulsion from, rather than attraction to, the negatively-charged pipe surface. However, electrostatic attraction as a consequence of the formation of a diffuse double layer (Alexander and Johnson 1950) is a distinct possibility. Zobell (1943) has indicated that solid surfaces can adsorb organic nutrients and inorganic ions and suggests that the characteristic adsorption of bacteria to solid surfaces in low-nutrient media is related to this. The adsorption of cations to the pipe surface, forming a diffuse double layer, could provide not only electrostatic attraction for negatively-charged bacteria but also a favourable concentration of nutrient cations. An alternative mechanism of attachment to surfaces is the production of holdfast material. As Zobell (1943) points out, it is possible that cells are first adsorbed physically and later produce

holdfast material to give more permanent binding. Most sessile, strongly-attaching bacteria appear to secrete a mucilaginous holdfast (Zobell, 1943) and in this context the holdfast material associated with Hyphomicrobium rosettes (Conti and Hirsch, 1965) may be of significance. The observation of hyphomicrobia apparently adsorbed to a fungal mycelium by the cell apex is further evidence that a mechanism such as secretion of holdfast material, as in rosette formation, may account for the attachment of hyphomicrobia to a pipe surface. Whatever the mechanism of adherence, it is clear that manganese-oxidizing hyphomicrobia eventually dominate the pipe surface even though it may be colonized initially by a great variety of microorganisms. This slection of a particular micro-organism bears some resemblance to the phenomenon of "take-over" in continuous cultures of E. coli, described by Munson and Bridges (1964). There, a mutant cell type arose which was able to adhere to the culture vessel, rapidly attaining dominance. Munson and Bridges suggest that adherence of cells to a surface is reversible and that "take-over" by a particular organism results from a multiplication rate exceeding the rate of detachment from the surface. Thus the manganese-oxidizing hyphomicrobia must either adhere to the pipe surface more firmly than other organisms or be capable of more rapid multiplication

in a low-nutrient environment. In pure culture, even in minimal media, some other bacteria always multiply more rapidly than hyphomicrobia and it seems that superior powers of adherence are more likely to account for the eventual dominance of this type of bacterium. In this connection, it is instructive to consider a pipeline as an elongated continuous-culture vessel into which dilute culture medium (lake water) is fed at a constant rate. Such a consideration easily explains the apparent anomaly of the high manganese concentration in deposits in pipelines carrying waters where manganese levels are at the limit of detection. Microbial oxidation and precipitation of manganese within the water itself, during passage through the pipeline, would be negligible because of the very low nutrient levels and high flow rates. However, an adsorbed bacterial flora would be able to take advantage of the continuous renewal of the dilute medium and large amounts of manganese could be deposited in the course of time.

The question of just where in a mineral deposit the causative microbes may live is posed by Ehrlich (1963a). To remain viable, they must inhabit fissures or pores in the deposit in order to allow for free movement of metabolites. An additional point is that a mineral deposit brought about by bacteria would tend to prevent multiplication simply by imposing physical restriction and it is in this context

that the curious morphology of stalked, budding bacteria in manganese deposits may hold special significance (Tyler and Marshall, 1967d).

In "conventional", rod-shaped bacteria an encrusting deposit would presumably impose very severe limitations and it is difficult to imagine continued reproduction taking place once a deposit was formed about the entire cell. Even in the chlamydobacteria, which commonly are implicated in manganese deposition, growth would be possible only at the free, unencrusted ends of the sheath. Romano and Geason (1964) have shown that growth in chlamydobacteria is limited to linear extension of the terminal part of the sheath, even when there is no restriction by encrustation. Aristovakaya (1963), however, suggests that the colonial organization of manganese-oxidizing hyphomicrobia is the most expedient form of existence in an encrusting environment. This intriguing idea does relate the form of these bacteria to their function in the deposition of manganese and provides an explanation for the build-up of deposits following establishment of the bacteria on the pipe surface. Aristovskaya envisages the formation of a bud at the end of a long stalk as a means for escape from the manganese deposit which is tending to isolate older cells from the medium. However, cells located in the central part of the colony may continue to metabolize, the essential nutrients being provided by

means of the cellular connection with younger, unencrusted parts of the colony. As manganese deposition spreads to the daughter cell the budding process could be repeated, ensuring the maintenance of a high metabolic and reproductive rate. This pattern of development is shown diagramatically in Fig. 48 where it is contrasted with the situation for chlamydobacteria and "conventional" bacteria. That this model for build-up of deposit is feasible is supported by the fact that Hyphomicrobium T37 habitually grows in the colonial form when oxidizing manganese, both in culture and in the pipelines. The occurrence of the colonial organization has been amply demonstrated by light- and electron-microscopy. Further support for the model comes from the behaviour of manganese-oxidizing hyphomicrobia in agar culture. The appearance of the edge of the colony suggests that the central mass of oxidized manganese is produced by confluence of the satellite centres, beyond which lie the ultimate, unencrusted daughter cells. Thus there is the potential for radial spread in three dimensions. Such a process would provide an ideal mechanism for the continuing build up of deposit on the pipe surface. Further, such a system of colonial development is self-perpetuating and endows on this particular microbial ecosystem a stability similar to that found in many macroecosytems. So stable a population is unusual for microbial ecosystems

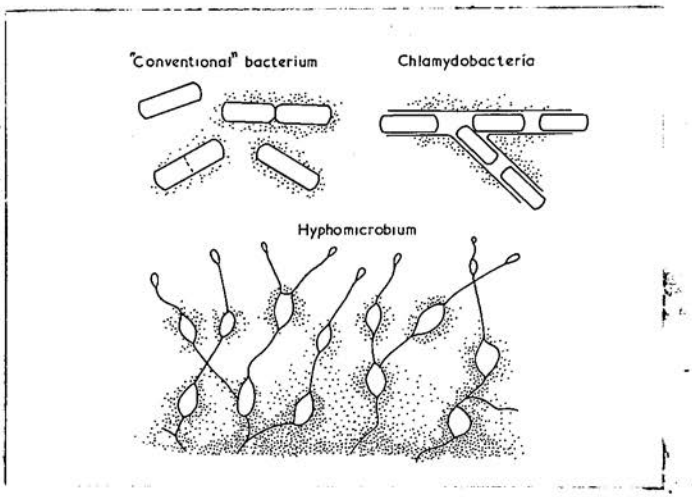


Fig. 48. Diagramatic comparison of manganese-oxidation by "conventional bacteria", chlamydobacteria and Hyphomicrobium, showing the comparative advantage of reproduction by budding.

(Brock, 1966); it is analogous to a climax vegetation.

In the present investigation no positive findings on the mechanism of microbial oxidation of manganese have been made. However, the study has suggested several possibilities for future work on this problem. The possibility that Hyphomicrobium is utilizing manganese chemoautotrophically is remote since the organism can grow without oxidizing manganese and on media where manganese is present in trace quantities. As the problem is most acute in Lake King William it seems that the humic waters draining from that catchment are the likely source of soluble manganese. In this case it is a likely possibility that the bacteria may release the manganese radicle from manganese chelates. However, the addition of manganese sulphate to Great Lake water produced a deposit of manganese oxides in the recirculatory apparatus, suggesting that manganous sulphate can be oxidized directly. Before progress on this aspect of the problem can be made it will be necessary to find a means of growing Hyphomicrobium in pure culture in such a way that it does not lose its ability to oxidize manganese.

This investigation has left many problems unsolved but, by surveying the problem in its broadest aspects, it has shown up those areas of the problem upon which attention should be focussed for future investigations in a long-term

project. As the problem is of considerable economic importance, future research will concentrate on aspects likely to lead to control measures. There are three major lines along which research should be directed:

a) the mechanism by which Hyphomicrobium attaches to the pipeline wall and the way in which it attains dominance over all other adherent bacteria. An understanding of this aspect may lead to control by preventing adsorption of the hyphomicrobia.

b) the form in which the available manganese exists in the water and the precise physiological mechanisms by which bacteria oxidize and precipitate this manganese.

c) the source of manganese in catchments or lakes and the mechanism by which it is brought into solution. The possibilities of control by catchment management or controlled limnological regimes should not be overlooked.

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